9/94 2098

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NEWS	2		"Ask CAS" for self-help around the clock
NEWS	3	SEP 09	CA/CAplus records now contain indexing from 1907 to the present
NEWS	4	Jul 15	Data from 1960-1976 added to RDISCLOSURE
NEWS	5	Jul 21	Identification of STN records implemented
NEWS	6	Jul 21	Polymer class term count added to REGISTRY
NEWS	7	Jul 22	INPADOC: Basic index (/BI) enhanced; Simultaneous Left and Right Truncation available
NEWS	8	AUG 05	New pricing for EUROPATFULL and PCTFULL effective August 1, 2003
NEWS	9	AUG 13	Field Availability (/FA) field enhanced in BEILSTEIN
NEWS	10	AUG 15	PATDPAFULL: one FREE connect hour, per account, in September 2003
NEWS	11	AUG 15	PCTGEN: one FREE connect hour, per account, in September 2003
NEWS	12	AUG 15	RDISCLOSURE: one FREE connect hour, per account, in September 2003
NEWS	13	AUG 15	TEMA: one FREE connect hour, per account, in September 2003
NEWS	14	AUG 18	Data available for download as a PDF in RDISCLOSURE
NEWS	15	AUG 18	Simultaneous left and right truncation added to PASCAL
NEWS	16	AUG 18	FROSTI and KOSMET enhanced with Simultaneous Left and Righ Truncation
NEWS	17	AUG 18	Simultaneous left and right truncation added to ANABSTR
NEWS	18	SEP 22	DIPPR file reloaded
NEWS	19	SEP 25	INPADOC: Legal Status data to be reloaded

NEWS EXPRESS	April 4 CURRENT WINDOWS VERSION IS V6.01a, CURRENT MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP), AND CURRENT DISCOVER FILE IS DATED 01 APRIL 2003
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=> e steward lance e/au
E1            2     STEWARD L M/AU
E2           13     STEWARD L R/AU
E3           14 --> STEWARD LANCE E/AU
E4            1     STEWARD LANCE EDWARD/AU
E5            1     STEWARD LAWRENCE R/AU
E6            2     STEWARD LAWRENCE RUSEELL/AU
E7            1     STEWARD LAWRENCE RUSSELL/AU
E8            2     STEWARD LESLIE A/AU
E9            2     STEWARD LESLIE ANN/AU
E10           1     STEWARD LIBBY L/AU
E11          21     STEWARD LUCINDA J/AU
E12           1     STEWARD LYNNE R/AU

=> s e3-e4
L1           15 ("STEWARD LANCE E"/AU OR "STEWARD LANCE EDWARD"/AU)

=> e fernandez-salas ester/au
E1            6     FERNANDEZ ZURBANO PURIFICACION/AU
E2           11     FERNANDEZ ZURITA C/AU
E3            0 --> FERNANDEZ-SALAS ESTER/AU
E4            1     FERNANDEZA/AU
E5            1     FERNANDEZA DE HENESTROSA A R/AU
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E7            2     FERNANDEZA J M/AU
E8            1     FERNANDEZA M R/AU
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E12           1     FERNANDEZB M/AU

=> e fernandez salas ester/au
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                Z SALAS ESTER"/AU OR "FERNANDEZ SALAS ESTER A"/AU OR "FERNANDEZ
                SALAS ESTHER"/AU)

=> e aoki kei roger/au
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E2            1     AOKI KEI R/AU
E3           24 --> AOKI KEI ROGER/AU
E4           19     AOKI KEIGO/AU
E5          136     AOKI KEIICHI/AU
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E11          11     AOKI KEIKICHI/AU
E12          33     AOKI KEIKO/AU

=> s e2-e3
L3           25 ("AOKI KEI R"/AU OR "AOKI KEI ROGER"/AU)

=> e aoki k r/au
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E2            1     AOKI K N/AU
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E12          62     AOKI KANAME/AU

=> s e3-e4
L4          125 ("AOKI K R"/AU OR "AOKI K ROGER"/AU)

=> s l1-l4
L5          211 (L1 OR L2 OR L3 OR L4)

=> s l5 and bont/a
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=> s l5 and botulinum (10a) fluorophore
L6            3 L5 AND BOTULINUM (10A) FLUOROPHORE

=> d bib ab1-3
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L6   ANSWER 1 OF 3  WPIDS  COPYRIGHT 2003 THOMSON DERWENT on STN
AN   2003-290198 [28]   WPIDS
DNC  C2003-075494
TI   Botulinum serotype A/E substrate useful for assaying protease activity of
     botulinum toxins, comprises donor fluorophore, acceptor
     and a clostridial toxin recognition sequence that includes a cleavage
     site.
DC   B04 D16
IN   AOKI, K R; FERNANDEZ-SALAS, E; STEWARD, L E
PA   (AOKI-I) AOKI K R; (FERN-I) FERNANDEZ-SALAS E; (STEW-I) STEWARD L E;
     (ALLR) ALLERGAN INC
CYC  100
PI   WO 2003020948 A2 20030313 (200328)* EN  168p    C12Q000-00
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            MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
            KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
            RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
     US 2003143650 A1 20030731 (200354)              G01N033-554
ADT  WO 2003020948 A2 WO 2002-US27145 20020822; US 2003143650 A1 US 2001-942024
     20010828
PRAI US 2001-942024   20010828
IC   ICM  C12Q000-00; G01N033-554
     ICS  C07K014-33; C12Q001-37; G01N033-569


=> d bib ab 1-3

L6   ANSWER 1 OF 3  WPIDS  COPYRIGHT 2003 THOMSON DERWENT on STN
AN   2003-290198 [28]   WPIDS
DNC  C2003-075494
TI   Botulinum serotype A/E substrate useful for assaying protease activity of
     botulinum toxins, comprises donor fluorophore, acceptor
     and a clostridial toxin recognition sequence that includes a cleavage
     site.
DC   B04 D16
IN   AOKI, K R; FERNANDEZ-SALAS, E; STEWARD, L E
PA   (AOKI-I) AOKI K R; (FERN-I) FERNANDEZ-SALAS E; (STEW-I) STEWARD L E;
     (ALLR) ALLERGAN INC
CYC  100
PI   WO 2003020948 A2 20030313 (200328)* EN  168p
        RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
            MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
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KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
US 2003143650 A1 20030731 (200354)
ADT WO 2003020948 A2 WO 2002-US27145 20020822; US 2003143650 A1 US 2001-942024 20010828
PRAI US 2001-942024 20010828
AB WO2003020948 A UPAB: 20030501
NOVELTY - A **botulinum** serotype A/E (BoNT/A/E) substrate, comprises a donor **fluorophore**, an acceptor having an absorbance spectrum overlapping the emission spectrum of donor fluorophore, and a BoNT A or BoNT/E recognition sequence comprising a cleavage site (the site intervenes between donor fluorophore and acceptor and under the appropriate conditions, resonance energy transfer is exhibited between the donor and acceptor).

USE - (I) is useful in assaying for the protease activity of any clostridial toxin, including botulinum toxins in a sample including bacterial, baculoviral and yeast lysate, raw, cooked or processed foods, beverages, animal feed, soil samples, water samples, cosmetics, tissue samples, and food or beverage sample. (I) is useful to assay a sample from a human or animal, for e.g., exposed to a clostridial toxin, or having one or more symptoms of a clostridial toxin, to follow activity during production and purification of clostridial toxin, and to assay formulated clostridial toxin products, including pharmaceuticals and cosmetics.

ADVANTAGE - The botulinum toxin substrates are utilized in rapid and simple homogenous screening assays that do not require separation of cleaved product from uncleaved substrate and do not rely on toxicity to animals.

Dwg.0/7

L6 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
AN 2003-13448 BIOTECHDS
TI Botulinum serotype A/E substrate useful for assaying protease activity of **botulinum** toxins, comprises donor **fluorophore**, acceptor and a clostridial toxin recognition sequence that includes a cleavage site;
botulinum toxin protease activity analysis in bacterium, baculo virus, yeast lysate, food, beverage, feedstuff, soil, water, cosmetic and tissue sample
AU STEWARD L E; **FERNANDEZ-SALAS E**; **AOKI K R**
PA ALLERGAN INC
PI WO 2003020948 13 Mar 2003
AI WO 2002-US27145 22 Aug 2002
PRAI US 2001-942024 28 Aug 2001; US 2001-942024 28 Aug 2001
DT Patent
LA English
OS WPI: 2003-290198 [28]
AB DERWENT ABSTRACT:
NOVELTY - A **botulinum** serotype A/E (BoNT/A/E) substrate, comprises a donor **fluorophore**, an acceptor having an absorbance spectrum overlapping the emission spectrum of donor fluorophore, and a BoNT A or BoNT/E recognition sequence comprising a cleavage site (the site intervenes between donor fluorophore and acceptor and under the appropriate conditions, resonance energy transfer is exhibited between the donor and acceptor).

WIDER DISCLOSURE - Also disclosed are: (1) BoNT/B, BoNT/C1, BoNT/D, BoNT/F, and BoNT/G substrates and their use for determining protease activity; (2) tetanus toxin (TeNT) substrate; (3) composite clostridial toxin substrate; and (4) kit for determining clostridial toxin protease activity in a sample.

BIOTECHNOLOGY - Preferred Substrate: (I) is a BoNT/A substrate and comprises a BoNT/A recognition sequence comprising a cleavage site, or is a BoNT/E substrate and comprises a BoNT/E recognition sequence comprising a cleavage site. (I) comprises at least 6 consecutive residues of

SNAP-25, comprising Gln-Arg (Gln(197)-Arg(198)) or Arg-Ile (Arg(180)-Ile(181)), or its peptidomimetic. (I) can be cleaved with an activity of at least 1, 20, 50, 100 or 150 nmol/minute/mg toxin. The acceptor is an acceptor fluorophore having a fluorescent lifetime of at least 1 microsecond. The acceptor is non-fluorescent. The donor fluorophore is fluorescein, Alexa Fluor (RTM), DABCYL, BODIPY. The acceptor is tetramethylrhodamine, EDANS, QSY (RTM) 7. The peptide or peptidomimetic has at most 20-100 residues. The donor fluorophore and acceptor fluorophore are separated by at most 15 residues, preferably 6 residues.

USE - (I) is useful in assaying for the protease activity of any clostridial toxin, including botulinum toxins in a sample including bacterial, baculoviral and yeast lysate, raw, cooked or processed foods, beverages, animal feed, soil samples, water samples, cosmetics, tissue samples, and food or beverage sample. (I) is useful to assay a sample from a human or animal, for e.g., exposed to a clostridial toxin, or having one or more symptoms of a clostridial toxin, to follow activity during production and purification of clostridial toxin, and to assay formulated clostridial toxin products, including pharmaceuticals and cosmetics.

ADVANTAGE - The botulinum toxin substrates are utilized in rapid and simple homogenous screening assays that do not require separation of cleaved product from uncleaved substrate and do not rely on toxicity to animals.

EXAMPLE - The fluorescent resonance energy transfer (FRET) substrate (A3) was synthesized by Alpha Diagnostics. X1-Asp-Ser-Asn-Lys-Thr-Arg-Ile-Asp-Glu-Ala-Asn- Gln-Arg-Ala-Thr-Lys-Met-Leu-Z2-NH2 (A3) This substrate contained a recognition sequence for BoNT/A flanked by a fluorescein-modified lysine residue (X1) and a tetramethylrhodamine-modified lysine residue (Z2) followed by a carboxy-terminal amide. Following proteolysis of botulinum toxin serotype A, the cleavage products (A4) were produced. X1-Asp-Ser-Asn-Lys-Thr-Arg-Ile-Asp-Glu-Ala-Asn-Gln and Arg-Ala-Thr-Lys-Met-Leu-Z2-NH2 (A4) Purified BoNT/A light chain (LC/A) or cellular extract containing LC/A was diluted in assay buffer. Dichain BoNT/A was incubated with 10 mM dithiothreitol (DTT) for about 30 minutes prior to analysis. Reactions contained various concentrations of LC/A, dichain toxin or formulated BOTOX (RTM) product, from 0.1 ng to 10 microg. Toxin was assayed. FRET substrate was added to a final concentration of 10 microM in a final volume of 100 microl assay buffer. The reaction is incubated at 37degreesC for 30 minutes, and was subsequently terminated by addition of 50 microl 2 M H2SO4. Fluorescence was measured with lambda(ex)= 488, lambda(Em) = 520 nm and lambda(em) = 585 nm. A reduction of at least 5% in the lambda(em) = 585 nm was indicative of BoNT/A protease activity. An increase of about 5% in the lambda(em) = 520 nm also was indicative of BoNT/A protease activity of the dichain or light chain botulinum toxin. These results demonstrated that botulinum toxin proteolytic activity can be assayed with an intramolecularly quenched FRET substrate.(168 pages)

L6 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:590711 CAPLUS
DN 139:129339
TI Fluorophore-labeled peptides and FRET assays for clostridial toxins
IN **Steward, Lance E.**; **Fernandez-Salas, Ester**; **Aoki, Kei Roger**
PA USA
SO U.S. Pat. Appl. Publ., 69 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI US 2003143651 A1 20030731 US 2001-942098 20010828
PRAI US 2001-942098 20010828
AB The present invention provides clostridial toxin substrates useful in assaying for the protease activity of any clostridial toxin, including botulinum toxins of all serotypes as well as tetanus toxins. A clostridial toxin substrate of the invention contains a donor fluorophore; an acceptor having an absorbance spectrum overlapping the emission spectrum of the donor fluorophore; and a clostridial toxin recognition sequence that includes a cleavage site, where the cleavage site intervenes between the donor fluorophore and the acceptor and where, under the appropriate conditions, resonance energy transfer is exhibited between the donor fluorophore and the acceptor.

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FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS, LIFESCI, CAPLUS' ENTERED AT 13:34:59 ON 28 SEP 2003
E STEWARD LANCE E/AU
L1 15 S E3-E4
E FERNANDEZ-SALAS ESTER/AU
E FERNANDEZ SALAS ESTER/AU
L2 65 S E1-E5
E AOKI KEI ROGER/AU
L3 25 S E2-E3
E AOKI K R/AU
L4 125 S E3-E4
L5 211 S L1-L4
L6 3 S L5 AND BOTULINUM (10A) FLUOROPHORE

=> s l5 and clostridial
L7 21 L5 AND CLOSTRIDIAL

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8 12 DUP REM L7 (9 DUPLICATES REMOVED)

=> d bib ab 1-12

L8 ANSWER 1 OF 12 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 1
AN 2003-290198 [28] WPIDS
DNC C2003-075494
TI Botulinum serotype A/E substrate useful for assaying protease activity of botulinum toxins, comprises donor fluorophore, acceptor and a **clostridial** toxin recognition sequence that includes a cleavage site.
DC B04 D16
IN **AOKI, K R**; **FERNANDEZ-SALAS, E**; STEWARD, L E
PA (AOKI-I) AOKI K R; (FERN-I) FERNANDEZ-SALAS E; (STEW-I) STEWARD L E; (ALLR) ALLERGAN INC
CYC 100
PI WO 2003020948 A2 20030313 (200328)* EN 168p
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
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US 2003143650 A1 20030731 (200354)
ADT WO 2003020948 A2 WO 2002-US27145 20020822; US 2003143650 A1 US 2001-942024 20010828

PRAI US 2001-942024 20010828
AB WO2003020948 A UPAB: 20030501
NOVELTY - A botulinum serotype A/E (BoNT/A/E) substrate, comprises a donor fluorophore, an acceptor having an absorbance spectrum overlapping the emission spectrum of donor fluorophore, and a BoNT A or BoNT/E recognition sequence comprising a cleavage site (the site intervenes between donor fluorophore and acceptor and under the appropriate conditions, resonance energy transfer is exhibited between the donor and acceptor).

USE - (I) is useful in assaying for the protease activity of any **clostridial** toxin, including botulinum toxins in a sample including bacterial, baculoviral and yeast lysate, raw, cooked or processed foods, beverages, animal feed, soil samples, water samples, cosmetics, tissue samples, and food or beverage sample. (I) is useful to assay a sample from a human or animal, for e.g., exposed to a **clostridial** toxin, or having one or more symptoms of a **clostridial** toxin, to follow activity during production and purification of **clostridial** toxin, and to assay formulated **clostridial** toxin products, including pharmaceuticals and cosmetics.

ADVANTAGE - The botulinum toxin substrates are utilized in rapid and simple homogenous screening assays that do not require separation of cleaved product from uncleaved substrate and do not rely on toxicity to animals.

Dwg.0/7

L8 ANSWER 2 OF 12 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 2
AN 2003-466155 [44] WPIDS
CR 2002-241566 [29]
DNC C2003-124296
TI Novel modified neurotoxin with a structural modification that alters biological persistence or activity of the modified neurotoxin relative to the unmodified neurotoxin, for treating tremors, bruxism and dysphagia.
DC B04 D16 D21
IN **AOKI, K R**; **FERNANDEZ-SALAS, E**; HERRINGTON, T M; STEWARD, L E
PA (ALLR) ALLERGAN SALES INC
CYC 1
PI US 2003027752 A1 20030206 (200344)* 33p
ADT US 2003027752 A1 CIP of US 2000-620840 20000721, US 2001-910346 20010720
PRAI US 2001-910346 20010720; US 2000-620840 20000721
AB US2003027752 A UPAB: 20030710
NOVELTY - A modified neurotoxin (I) comprising a structural modification alters the biological persistence or biological activity of the modified neurotoxin relative to an identical neurotoxin without the structural modification (the modified neurotoxin is structurally different from a naturally existing neurotoxin), is new.

DETAILED DESCRIPTION - A modified neurotoxin (I) comprising a structural modification. The structural modification alters a biological persistence or biological activity of the modified neurotoxin relative to an identical neurotoxin without the structural modification. The modified neurotoxin is structurally different from a naturally existing neurotoxin. (I) comprises a neurotoxin including a structural modification effective to alter the biological persistence of the modified neurotoxin relative to an identical neurotoxin without the structural modification. The neurotoxin comprises the amino acid sequence regions:

(a) a first region effective as a cellular binding moiety;

(b) a second region effective to translocate a modified neurotoxin or its part across an endosome membrane; and

(c) a third region effective to inhibit exocytosis when released into a cytoplasm of a target cell (at least one of the first, second and third regions is substantially derived from a **Clostridial** neurotoxin, and the third region includes the structural modification).

INDEPENDENT CLAIMS are also included for:

(1) enhancing biological persistence of (I), involves fusing or adding structural modification to neurotoxin;

(2) reducing biological persistence of neurotoxin, by mutating amino acid of neurotoxin; and

(3) a modified neurotoxin comprising a botulinum type A neurotoxin including a structural modification which is effective to alter a biological persistence of the modified neurotoxin relative to an identical neurotoxin without the structural modification, where the modification comprises a deletion of amino acids 1-8 and 416-437 or comprises substitution of leucine at position 427 for an alanine and leucine at position 428 for alanine from a light chain of the neurotoxin.

ACTIVITY - Analgesic; Antiasthmatic; Antiinflammatory.

A 76 year old man who presented a post-therapeutic type pain localized to the abdomen region, was treated by a bolus injection of a modified neurotoxin intradermally to the abdomen, the modified neurotoxin was for e.g. botulinum type A, B, C1, C2, D, E, F and/or G. The modified neurotoxin comprised a leucine-based motif and/or additional tyrosine-based motifs. Within 1-7 days after modified neurotoxin administration the patient's pain was substantially alleviated. The duration of the pain alleviation was from 7-27 months.

MECHANISM OF ACTION - Inhibits release of Neurosubstances e.g. substance P from the peripheral primary sensory terminal by inhibiting SNARE-dependent exocytosis; Dampens transmission of pain signals from reaching the brain.

USE - (I) which has altered biological persistence, is useful for treating a condition in a mammal, where the neurotoxin does not comprise a leucine-based motif, and the structural modification includes a biological persistence enhancing component which comprises a leucine-based motif, tyrosine-based motif or an amino acid derivative. (I) is useful for treating a condition such as neuromuscular disorder, autonomic disorder or pain, or spasmodic dysphonia, laryngeal dystonia, oromandibular dysphonia, lingual dystonia, cervical dystonia, focal hand dystonia, blepharospasm, strabismus, hemifacial spasm, eyelid disorder, cerebral palsy, focal spasticity, spasmodic colitis, neurogenic bladder, anismus, limb spasticity, tics, tremors, bruxism, anal fissure, achalasia, dysphagia, lacrimation, hyperhydrosis, excessive salivation, excessive gastrointestinal secretions, pain from muscle spasms, headache pain, brow furrows and skin wrinkles (claimed).

(I) is also useful for treating spinal curvature, various forms of inflammatory pains, autonomic nervous system disorders, e,g., an respiratory malfunctioning such as chronic obstructive pulmonary disease, and asthma; pain not associated with a muscular disorder, such as spasm.

ADVANTAGE - An unit amount of modified neurotoxin having altered biological activity than natural neurotoxin, is more efficient to reduce exocytosis from a cell than is a unit amount of naturally existing neurotoxin (claimed).

Dwg.0/10

L8 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:590711 CAPLUS
DN 139:129339
TI Fluorophore-labeled peptides and FRET assays for **clostridial** toxins
IN **Steward, Lance E.**; **Fernandez-Salas, Ester**; **Aoki, Kei Roger**
PA USA
SO U.S. Pat. Appl. Publ., 69 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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PI US 2003143651 A1 20030731 US 2001-942098 20010828
PRAI US 2001-942098 20010828
AB The present invention provides **clostridial** toxin substrates useful in assaying for the protease activity of any **clostridial** toxin, including botulinum toxins of all serotypes as well as tetanus toxins. A **clostridial** toxin substrate of the invention contains a donor fluorophore; an acceptor having an absorbance spectrum overlapping the emission spectrum of the donor fluorophore; and a **clostridial** toxin recognition sequence that includes a cleavage site, where the cleavage site intervenes between the donor fluorophore and the acceptor and where, under the appropriate conditions, resonance energy transfer is exhibited between the donor fluorophore and the acceptor.

L8 ANSWER 4 OF 12 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 3
AN 2002-479904 [51] WPIDS
DNC C2002-136605
TI Modified neurotoxin especially **Clostridial** toxins, useful for treating neuromuscular and autonomic nervous system disorder and pain, comprises structural modification to alter biological persistence of neurotoxin.
DC B04 C03 D16
IN **AOKI, K R**; LIN, W; SPANOYANNIS, A; STEWARD, L E
PA (ALLR) ALLERGAN SALES INC; (ALLR) ALLERGAN INC
CYC 98
PI WO 2002040506 A2 20020523 (200251)* EN 55p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2002019850 A 20020527 (200261)
US 2002127247 A1 20020912 (200262)
EP 1334120 A2 20030813 (200355) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
ADT WO 2002040506 A2 WO 2001-US44030 20011116; AU 2002019850 A AU 2002-19850 20011116; US 2002127247 A1 Provisional US 2000-249540P 20001117, US 2001-4230 20011031; EP 1334120 A2 EP 2001-996547 20011116, WO 2001-US44030 20011116
FDT AU 2002019850 A Based on WO 2002040506; EP 1334120 A2 Based on WO 2002040506
PRAI US 2000-249540P 20001117; US 2001-4230 20011031
AB WO 200240506 A UPAB: 20020812
NOVELTY - A modified neurotoxin especially **Clostridial** botulinum toxins (I) comprising a neurotoxin including a structural modification, which is effective to alter (increase or decrease) the biological persistence of (I), relative to an identical neurotoxin without the modification, where (I) is structurally different from a naturally occurring neurotoxin, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for making (I), which comprises producing a polypeptide from an oligonucleotide having codes for a neurotoxin including a structural modification.

ACTIVITY - Analgesic; Antiasthmatic. (I) was tested for its analgesic activity. An unfortunate 36 year old woman had a 15 year history of temporomandibular joint disease and chronic pain along the masseter and temporalis muscles. She was diagnosed as having temporomandibular joint (TMJ) dysfunction with subluxation of the joint and was treated with surgical orthoplasty meniscusectomy and condyle resection. She continued to have difficulty with opening and closing her jaw after the surgical procedures and for this reason, several years later, a surgical procedure to replace prosthetic joints on both sides was performed. She was

diagnosed as having post-surgical myofascial pain syndrome and was injected with about 8-15 U/kg of (I) into the masseter and temporalis muscles, preferably the modified neurotoxin comprises botulinum toxin serotype (BoNT)/E with an N-terminal myristylation site, e.g., Gly-Val-Asp-Ile-Ala-Tyr, fused to position 15 of its light chain, or a position substantially corresponding to position 15 of the BoNT/A light chain. Several days after the injections she noted substantial improvement in her pain and reports that her jaw feels looser. This gradually improved over a 2-3 weeks period in which she noted increased ability to open the jaw and a diminishing pain. The patient stated that the pain was better than at any time in the last 4 years. The improved condition persisted for up to 27 months after the original injection of (I).

MECHANISM OF ACTION - Affects the ability of a degrading protease to act directly on the molecule and/or affect the ability of the molecules to be sequestered into vesicles to be protected against these degrading proteases.

USE - (I) is useful for treating a biological disorder which include neuromuscular e.g. strabismus, blepharospasm, spasmodic torticollis (cervical dystonia), oromandibular dystonia and spasmodic dysphonia (laryngeal dystonia), autonomic nervous system disorders e.g. excessive salivation and sweating, asthma etc. and pain e.g. headache, muscular tension, neuralgia and neuropathy.

Dwg.0/0

L8 ANSWER 5 OF 12 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 4
AN 2002-241566 [29] WPIDS
CR 2003-466155 [44]
DNC C2002-072658
TI Novel modified neurotoxin comprising structural modification which alters the biological persistence and/or biological activity of a neurotoxin, useful for treating neuromuscular or autonomic disorder, or pain.
DC B04
IN **AOKI, K R**; **FERNANDEZ-SALAS, E**; HERRINGTON, T M; STEWARD, L E
PA (ALLR) ALLERGAN SALES INC; (ALLR) ALLERGAN INC
CYC 97
PI WO 2002008268 A2 20020131 (200229)* EN 102p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2001080703 A 20020205 (200236)
EP 1309618 A2 20030514 (200333) EN
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
BR 2001012715 A 20030520 (200342)
KR 2003033000 A 20030426 (200354)
ADT WO 2002008268 A2 WO 2001-US23122 20010720; AU 2001080703 A AU 2001-80703 20010720; EP 1309618 A2 EP 2001-959115 20010720, WO 2001-US23122 20010720; BR 2001012715 A BR 2001-12715 20010720, WO 2001-US23122 20010720; KR 2003033000 A KR 2003-700901 20030121
FDT AU 2001080703 A Based on WO 2002008268; EP 1309618 A2 Based on WO 2002008268; BR 2001012715 A Based on WO 2002008268
PRAI US 2000-620840 20000721
AB WO 200208268 A UPAB: 20030821
NOVELTY - A modified neurotoxin (NT) (I) comprising NT including a structural modification, where the structural modification is effective to alter a biological persistence of NT, or biological activity of NT, relative to identical NT without the structural modification, is new. (I) is structurally different from a naturally existing NT.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for

reducing (M1) the biological persistence of NT by mutating an amino acid of the NT.

ACTIVITY - Analgesic; Neuroprotective; Antiinflammatory.

A 46 year old woman who presented a shoulder-hand syndrome type, was treated by a bolus injection of a modified neurotoxin subcutaneously to the shoulder. The modified neurotoxin is botulinum type E comprising a leucine-based motif. The modified neurotoxin can also be, for example, modified botulinum type A, B, C1, C2, D, E, F or G which comprised a leucine-based motif. Within 1-7 days after modified neurotoxin administration the patient's pain was substantially alleviated. The duration of the pain alleviation was 7-27 months.

MECHANISM OF ACTION - Release of substance P from peripheral primary sensory terminal inhibitor by inhibiting SNARE-dependent exocytosis.

USE - (I)(a) is useful for treating a condition e.g. neuromuscular disorder, autonomic disorder or pain in a mammal, where the NT does not comprise a leucine-based motif, and the structural modification includes a biological persistence enhancing component which comprises a leucine-based or tyrosine-based motif, or an amino acid derivative. (I)(a) is thus useful for treating spasmodic dysphonia, laryngeal dystonia, oromandibular dysphonia, lingual dystonia, cervical dystonia, focal hand dystonia, blepharospasm, strabismus, hemifacial spasm, eyelid disorder, cerebral palsy, focal spasticity, spasmodic colitis, neurogenic bladder, anismus, limb spasticity, tics, tremors, bruxism, anal fissure, achalasia, dysphagia, lacrimation, hyperhydrosis, excessive salivation, excessive gastrointestinal secretions, pain from muscle spasms, headache pain, brow furrows or skin wrinkles. (All claimed). (I) is useful for treating spinal curvature. (I) is useful for treating an autonomous nervous system disorder including respiratory malfunctioning such as chronic obstructive pulmonary disease and asthma. (I) is useful for treating muscular tension, neuralgia or neuropathy.

ADVANTAGE - (I) has enhanced or decreased biological persistence and/or biological half life and/or enhanced or decreased biological activity as compared to unmodified NT. The biological half-life and/or the biological activity of the (I) is enhanced by 100 %. (I) including the biological persistence enhancing component is able to cause a substantial inhibition of neurotransmitter release e.g. acetylcholine from a nerve terminal for 20-300 % longer than a NT that is not modified.

Dwg.0/10

L8 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:521523 CAPLUS
DN 137:73273
TI Adrenergic receptor ligand-neurotoxin conjugates and methods for treating pain
IN Gil, Daniel W.; **Aoki, Kei Roger**
PA Allergan Sales, Inc., USA
SO PCT Int. Appl., 76 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002053177	A2	20020711	WO 2001-US48651	20011214

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI US 2000-751053 A 20001229
OS MARPAT 137:73273
AB Agents for treating pain, methods for producing the agents, and methods for treating pain by administration to a patient of a therapeutically effective amt. of the agent, are disclosed. The agent may include a **clostridial** neurotoxin, a fragment or a deriv. thereof, attached to a targeting component, wherein the targeting component is selected form a group consisting of compds. which selectively binds at the .alpha.2b or .alpha.2b/.alpha.2c adrenergic receptor subtype(s) as compared to other binding sites, e.g. the .alpha.2a adrenergic receptor subtype.

L8 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2002:519991 BIOSIS
DN PREV200200519991
TI Retargeted **clostridial** endopeptidase: Antinociceptive activity in preclinical models of pain.
AU Cui, M. (1); Chaddock, J. A.; Rubino, J. (1); Khanijou, S. (1); Duggan, M. J.; Walsh, B.; Foster, K. A.; **Aoki, K. R. (1)**
CS (1) Allergan Inc., 2525 Dupont Drive, Irvine, CA, 92612 USA
SO Naunyn-Schmiedeberg's Archives of Pharmacology, (June, 2002) Vol. 365, No. Supplement 2, pp. R16. print.
Meeting Info.: International Conference on Basic and Therapeutic Aspects of Botulinum and Tetanus Toxins Hannover, Germany June 08-12, 2002
ISSN: 0028-1298.
DT Conference
LA English

L8 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2002:519986 BIOSIS
DN PREV200200519986
TI Retargeted **clostridial** endopeptidases: Inhibition of nociceptive neurotransmitter release in vitro, and antinociceptive activity in in vivo models of pain.
AU Chaddock, J. A. (1); Duggan, M. J. (1); Hall, Y. H. J. (1); Kirby, E. R. (1); Moulsdale, H. J. (1); Purkiss, J. R. (1); Quinn, C. P. (1); Shone, C. C. (1); Dickenson, A. H.; Cui, M.; **Aoki, K. R.**; Foster, K. A. (1)
CS (1) Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 0JG UK
SO Naunyn-Schmiedeberg's Archives of Pharmacology, (June, 2002) Vol. 365, No. Supplement 2, pp. R15. print.
Meeting Info.: International Conference on Basic and Therapeutic Aspects of Botulinum and Tetanus Toxins Hannover, Germany June 08-12, 2002
ISSN: 0028-1298.
DT Conference
LA English

L8 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:435285 BIOSIS
DN PREV200100435285
TI Modification of **clostridial** toxins for use as transport proteins.
AU Dolly, James Oliver (1); **Aoki, Kei Roger**; Wheeler, Larry Allen; Garst, Michael Elwood
CS (1) Cheam UK
ASSIGNEE: Allergan Sales, Inc.
PI US 6203794 March 20, 2001
SO Official Gazette of the United States Patent and Trademark Office Patents, (Mar. 20, 2001) Vol. 1244, No. 3, pp. No Pagination. e-file.
ISSN: 0098-1133.
DT Patent
LA English
AB A chemical conjugate for treating a nerve cell related disorder is

provided. The conjugate includes an active or inactive **Clostridial** toxin having specificity for a target nerve cell. The toxin is conjugated to a drug or other bioactive molecule without affecting the toxin's ability to enter the target nerve cell.

L8 ANSWER 10 OF 12 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
AN 2001-502158 [55] WPIDS
CR 2000-610759 [51]; 2002-178612 [08]
DNC C2001-150983
TI Treatment of pain e.g. inflammatory pain involves intraspinal administration of a neurotoxin to a mammal.
DC B04
IN **AOKI, K R**; CUI, M
PA (AOKI-I) AOKI K R; (CUIM-I) CUI M; (ALLR) ALLERGAN SALES INC
CYC 1
PI US 2001012828 A1 20010809 (200155)* 20p
US 6372226 B2 20020416 (200232)
ADT US 2001012828 A1 Cont of US 1999-417195 19991012, Cont of US 2000-578097 20000525, US 2001-797556 20010301; US 6372226 B2 Cont of US 1999-417195 19991012, Cont of US 2000-578097 20000525, US 2001-797556 20010301
FDT US 2001012828 A1 Cont of US 6113915, Cont of US 6235289; US 6372226 B2 Cont of US 6113915, Cont of US 6235289
PRAI US 1999-417195 19991012; US 2000-578097 20000525; US 2001-797556 20010301
AB US2001012828 A UPAB: 20020521
NOVELTY - Treatment of pain or in vivo attenuation of a nociceptive activity or experience of a human patient involves the step of intraspinal administration of neurotoxin (preferably botulinum) to a mammal. Neurotoxin is free of any neuronal targeting group.

ACTIVITY - Analgesic; Antiinflammatory.

A patient, age 51, experiencing pain subsequent to injury to his hand, arm, foot or leg was treated by intrathecal administration e.g. by spinal, tap or by catheterization to the spinal cord, such as the lumbar region of the spinal cord, with botulinum toxin type A (0.1 - 30 U/kg). Within 1 - 7 days after toxin administration the patient's pain is subsequently alleviated.

MECHANISM OF ACTION - None given.

USE - In pharmaceutical preparation for the in vivo attenuation of a nociceptive activity (such as neuropathin pain syndrome and inflammatory pain) or experience of a human patient, for improving patient function and for treating pain (all claimed) such as pain subsequent to spinal cord injury or limb injury, pain associated with cancer and diabetes.

ADVANTAGE - There is improvement observed in at least one of factors of reduced pain, reduced time spent in bed, increased ambulation, healthier attitude and a more varied lifestyle, after intraspinal administration of neurotoxin. The administration of neurotoxin gives long duration of activity, low rates of diffusion out of an intrathecal space where administered, low rates of diffusion to other intrathecal areas outside of the site of administration. The method had limited or insignificant side effects at therapeutic dose levels. The method provides significant pain alleviation even though the neurotoxin is not administrated in conjunction with any non-native or non-inherent to the neurotoxin neuronal targeting moiety. By intraspinal neurotoxin administration the symptoms of pain can be dramatically reduced for 2 - 4 months per injection of neurotoxin and pain alleviating effect persists for up to 10 days (preferably 20 days, especially 3 months). The injected neurotoxin tends to exert a CNS (central nervous system) site specific antinociceptive effect. The amount of neurotoxin injected intraspinally can be considerably less than the amount of the same neurotoxin required by other routes of administration i.e. intramuscular intrasphincter, oral or parenteral to achieve a comparable effect.

Dwg.0/7

L8 ANSWER 11 OF 12 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 5
AN 2000-072166 [06] WPIDS
DNC C2000-020536
TI Novel methods and compositions for extending the action of **Clostridial** neurotoxin used for modulating neurite outgrowth in damaged neural endplates.
DC B04 D16
IN **AOKI, K R**; DE PAIVA, A; DOLLY, J O
PA (ALLR) ALLERGAN SALES INC
CYC 84
PI WO 9955359 A1 19991104 (200006)* EN 46p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZW
AU 9937484 A 19991116 (200015)
EP 1073455 A1 20010207 (200109) EN
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
JP 2002512977 W 20020508 (200234) 38p
ADT WO 9955359 A1 WO 1999-US8303 19990415; AU 9937484 A AU 1999-37484 19990415; EP 1073455 A1 EP 1999-919857 19990415, WO 1999-US8303 19990415; JP 2002512977 W WO 1999-US8303 19990415, JP 2000-545557 19990415
FDT AU 9937484 A Based on WO 9955359; EP 1073455 A1 Based on WO 9955359; JP 2002512977 W Based on WO 9955359
PRAI US 1998-83472P 19980429
AB WO 9955359 A UPAB: 20000203
NOVELTY - Compositions for extending the action of **Clostridial** neurotoxin are new.

DETAILED DESCRIPTION - A novel method for extending the effective period during which tissue treated with a **clostridial** toxin is paralysed comprises contacting the tissue with a composition comprises an agent able to prevent the neuroregenerative activity of a polypeptide selected from IGF I, IGF II, cilary neurotrophic factor, NT-3, NT-4, brain- derived neurotrophic factor, leukemia inhibitory factor, tenascin-C, ninjurin, neural cell adhesion molecule, and neural agrin.

An INDEPENDENT CLAIM is also included for a method for stimulating the outgrowth of neural sprouts from damaged neural tissue, comprising contacting the tissue with a composition comprising a polypeptide which comprises a neurotrophically active domain derived from an agent selected from IGF I, IGF II, cilary neurotrophic factor, NT-3, NT-4, brain-derived neurotrophic factor, leukemia inhibitory factor, tenascin-C, ninjurin, neural cell adhesion molecule, and neural agrin.

USE - Preventing the sprouting seen in neural endplates after treatment with **clostridial** neurotoxin (CN) results in the extension of the effective period during which tissue treated with toxin remains paralyzed. Blocking muscle derived diffusable factors positively affecting sprouting attenuates the effects of CN. To this end, novel compositions and methods increasing the effectiveness of treatment of tissue with CN.The methods can be used to inhibit or reduce the activity of a variety of nerve factors, e.g. neurotophins. The methods are used for extending the effective period during which tissue treated with **clostridial** toxin is paralysed. The methods may also be used for stimulating the outgrowth of neural sprouts from damaged neural tissue, e.g. to treat nerve or spinal cord crush injuries, traumatic brain injuries, glaucoma-induced damage to the retina and/or optic nerve, blepharospasm, stroke, multiple sclerosis, cerebral palsy, of or surgical trauma or injury.

ADVANTAGE - The methods of the invention increase the therapeutic life of the toxin (which is reduced by sprouting of nascent, synaptically active processes at the neuromuscular junction), and so lead to a concomitant lessening in the required frequency of treatment of the

patient with the neurotoxin. Reducing frequency of treatment would provide less opportunity for a patient to experience the side-effects of the toxin treatment. Also reduced frequency of treatment provides less opportunity for miscalculation of dosage amount and other treatment-specific risks. Dwg.0/0

L8 ANSWER 12 OF 12 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 6
AN 1996-030343 [03] WPIDS
DNC C1996-010402
TI New chemical conjugates of Chlostridial neurotoxin cpds. - used for targetting agents to nerve cells, for treating nerve cell related disorders, botulism or tetanus.
DC B04
IN **AOKI, K R**; DOLLY, J O; GARST, M E; WHEELER, L A
PA (ALLR) ALLERGAN INC; (ALLR) ALLERGAN SALES INC
CYC 65
PI WO 9532738 A1 19951207 (199603)* EN 68p
RW: AT BE CH DE DK ES FR GB GR IE IT KE LU MC MW NL OA PT SD SE SZ UG
W: AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TT UA UG US UZ VN
AU 9526222 A 19951221 (199612)
EP 760681 A1 19970312 (199715) EN
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
JP 10500988 W 19980127 (199814) 80p
AU 695623 B 19980820 (199845)
EP 760681 B1 19990901 (199940) EN
R: CH DE DK ES FR GB IE IT LI NL SE
DE 69511860 E 19991007 (199947)
ES 2138740 T3 20000116 (200011)
CA 2191754 C 20001212 (200103) EN
US 6203794 B1 20010320 (200118)
ADT WO 9532738 A1 WO 1995-GB1253 19950531; AU 9526222 A AU 1995-26222 19950531; EP 760681 A1 EP 1995-921007 19950531, WO 1995-GB1253 19950531; JP 10500988 W WO 1995-GB1253 19950531, JP 1996-500524 19950531; AU 695623 B AU 1995-26222 19950531; EP 760681 B1 EP 1995-921007 19950531, WO 1995-GB1253 19950531; DE 69511860 E DE 1995-611860 19950531, EP 1995-921007 19950531, WO 1995-GB1253 19950531; ES 2138740 T3 EP 1995-921007 19950531; CA 2191754 C CA 1995-2191754 19950531, WO 1995-GB1253 19950531; US 6203794 B1 WO 1995-GB1253 19950531, US 1997-750101 19970501
FDT AU 9526222 A Based on WO 9532738; EP 760681 A1 Based on WO 9532738; JP 10500988 W Based on WO 9532738; AU 695623 B Previous Publ. AU 9526222, Based on WO 9532738; EP 760681 B1 Based on WO 9532738; DE 69511860 E Based on EP 760681, Based on WO 9532738; ES 2138740 T3 Based on EP 760681; CA 2191754 C Based on WO 9532738; US 6203794 B1 Based on WO 9532738
PRAI GB 1994-10871 19940531; GB 1994-10870 19940531
AB WO 9532738 A UPAB: 19991122
A chemical conjugate for treating a nerve cell related disorder is claimed comprising: (a) an inactive **Clostridial** neurotoxin (CN) having specificity for a target nerve cell and (b) a drug or other bioactive molecule attached to the CN, where the CN retains its ability to enter the target nerve cell. Also claimed are: (1) the use of an inactive CN in the prepn. of a medicament for treatment of acute botulinum toxin poisoning; (2) use of a chemical conjugate comprising an active CN and a drug in the prepn. of a medicament for treatment of focal dystonias, spasticities due to stroke or traumatic brain or spinal cord injury, blepharospasm, strabismus, cerebral palsy or back pain due to muscle spasms; (3) a method for treating a neuromuscular dysfunction in a mammal comprising introducing a pharmaceutically active soln. comprising a CN linked to a drug into the mammal.

USE - The CN conjugates can be used to treat nerve cell related disorders or to treat botulism or tetanus.

ADVANTAGE - The CN conjugates have the ability to be effectively internalised and transported to the cytosol of cells. They can specifically deliver agents to target neurons.
Dwg.0/9

=> s bonta
L9 55 BONTA

=> s l9 and clostridial
L10 0 L9 AND CLOSTRIDIAL

=> s l9 and botulinum
L11 37 L9 AND BOTULINUM

=> s l11 and fluorophore
L12 0 L11 AND FLUOROPHORE

=> dup rem l11
PROCESSING COMPLETED FOR L11
L13 13 DUP REM L11 (24 DUPLICATES REMOVED)

=> d bib ab 1-13

L13 ANSWER 1 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
AN 2003:70288 BIOSIS
DN PREV200300070288
TI **Botulinum** neurotoxin A attenuates release of norepinephrine but not NPY from vasoconstrictor neurons.
AU Morris, Judy L. (1); Jobling, Phillip; Gibbins, Ian L.
CS (1) Dept. of Anatomy and Histology, Flinders Univ., GPO Box 2100, Adelaide, SA, 5001, Australia: judy.morris@flinders.edu.au Australia
SO American Journal of Physiology, (December 2002, 2002) Vol. 283, No. 6 Part 2, pp. H2627-H2635. print.
ISSN: 0002-9513.
DT Article
LA English
AB We examined effects of **botulinum** neurotoxin A (**BoNTA**) on sympathetic constrictions of the vena cava and uterine artery from guinea pigs to test the role of soluble NSF attachment protein receptor (SNARE) proteins in release of the cotransmitters norepinephrine (NE) and neuropeptide Y (NPY). Protein extracts of venae cavae and uterine arteries showed partial cleavage of synaptosomal associated protein of 25 kDa (SNAP-25) after treatment in vitro with **BoNTA** (50-100 nM). The rising phase of isometric contractions of isolated venae cavae to field stimulation at 20 Hz, mediated by NE acting on alpha-adrenoceptors, was reduced significantly by 100 nM **BoNTA**. However, sustained sympathetic contractions mediated by NPY were not affected by **BoNTA**. In uterine arteries, noradrenergic contractions to 1-Hz stimulation were almost abolished by **BoNTA**, and contractions at 10 Hz were reduced by 50-60%. We conclude that SNARE proteins are involved in exocytosis of NE from synaptic vesicles at low frequencies of stimulation but may not be essential for exocytosis of NPY and NE from large vesicles at high stimulation frequencies.

L13 ANSWER 2 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2
AN 2002:323883 BIOSIS
DN PREV200200323883
TI A pilot study to investigate the combined use of **botulinum** neurotoxin type A and functional electrical stimulation, with physiotherapy, in the treatment of spastic dropped foot in subacute

stroke.
AU Johnson, Catherine A. (1); Wood, Duncan E.; Swain, Ian D.; Tromans, Anthony M.; Strike, Paul; Burridge, Jane H.
CS (1) Clinical Research Physiotherapist, Department of Medical Physics and Biomedical Engineering, Salisbury District Hospital, Wiltshire, SP2 8BJ: calj@mpbe-sdh.demon.co.uk UK
SO Artificial Organs, (March, 2002) Vol. 26, No. 3, pp. 263-266. http://www.blackwell-science.com/ cgilib/bsinc.bin?Journal=artificial.print.
ISSN: 0160-564X.
DT Article
LA English
AB The objective was to inform sample size calculations for a full randomized controlled trial (RCT). The design included an RCT pilot trial with a 16 week study period, including a 4 week baseline phase. The subjects were adults within 1 year of first stroke, ambulant with a spastic dropped foot. Twenty-one participants were recruited from the stroke services of 4 centers. For intervention all participants received physiotherapy; the treatment group also received **botulinum** neurotoxin Type A (**BoNTA**) intramuscular injections to triceps surae (800 U Dysport) and functional electrical stimulation (FES) of the common peroneal nerve to assist walking. The main outcome measure was walking speed. The result was a significant upward trend in median walking speed for both the control ($p = 0.02$) and treatment groups (nonstimulated $p = 0.004$, stimulated $p = 0.042$). Trend lines were different in location ($p = 0.04$ and $p = 0.009$, respectively). In conclusion, there is evidence of an additional, beneficial effect of **BoNTA** and FES. Sufficient information has been gained on the variability of the primary outcome measure to inform sample size calculations for a full RCT to quantify the treatment effect with precision.

L13 ANSWER 3 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:399373 CAPLUS
DN 139:212528
TI Purification of recombinant protective fragment of **botulinum** neurotoxin serotype A by IEF
AU Wang, Hui; Yin, Jun
CS Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, 100071, Peop. Rep. China
SO Shengming Kexue Yanjiu (2002), 6(2), 133-136
CODEN: SKYAFL; ISSN: 1007-7847
PB Shengming Kexue Yanjiu Bianji Weiyuanhui
DT Journal
LA Chinese
AB Recombinant protective fragment (r BoNTaHc468) of **botulinum** neurotoxin serotype A (**BoNTa**) was expressed at high level in insol. form in E. coli. E. coli cell pellets were centrifuged after E. coli strain contg. recombinant expression plasmid pBV-BoNTaHc468 was activated, and inclusion body was made. The inclusion body was resolved with 8 mol/L deionized urea. The protein was finally purified through isoelectronic focus (IEF) prepn. electrophoresis. PI of protein was about pH 8.0-8.5. The purity of protein was over 90%. The purified protein was pos. in ELISA. The successful purifn. and expression of protective fragment (rBoNTaHc468) of **botulinum** neurotoxin serotype A is a key to further study on gene engineering antitoxin and subunit vaccine.

L13 ANSWER 4 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3
AN 2001:567265 BIOSIS
DN PREV200100567265
TI Differential inhibition by **botulinum** neurotoxin A of cotransmitters released from autonomic vasodilator neurons.
AU Morris, Judy L. (1); Jobling, Phillip; Gibbins, Ian L.

CS (1) Dept. of Anatomy and Histology, Flinders Univ., Adelaide, SA, 5001: Judy.Morris@flinders.edu.au Australia
SO American Journal of Physiology, (November, 2001) Vol. 281, No. 5 Part 2, pp. H2124-H2132. print.
ISSN: 0002-9513.
DT Article
LA English
SL English
AB The role of the soluble NSF attachment protein receptor (SNARE) protein complex in release of multiple cotransmitters from autonomic vasodilator neurons was examined in isolated segments of guinea pig uterine arteries treated with **botulinum** neurotoxin A (**BoNTA**; 50 nM). Western blotting of protein extracts from uterine arteries demonstrated partial cleavage of synaptosomal-associated protein of 25 kDa (SNAP-25) to a NH2-terminal fragment of apprx24 kDa by **BoNTA**. **BoNTA** reduced the amplitude (by 70-80%) of isometric contractions of arteries in response to repeated electrical stimulation of sympathetic axons at 1 or 10 Hz. The amplitude of neurogenic relaxations mediated by neuronal nitric oxide (NO) was not affected by **BoNTA**, whereas the duration of peptide-mediated neurogenic relaxations to stimulation at 10 Hz was reduced (67% reduction in integrated responses). In contrast, presynaptic cholinergic inhibition of neurogenic relaxations was abolished by **BoNTA**. These results demonstrate that the SNARE complex has differential involvement in release of cotransmitters from the same autonomic neurons: NO release is not dependant on synaptic vesicle exocytosis, acetylcholine release from small vesicles is highly dependant on the SNARE complex, and neuropeptide release from large vesicles involves SNARE proteins that may interact differently with regulatory factors such as calcium.

L13 ANSWER 5 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:60907 CAPLUS
DN 137:227237
TI Cloning and sequencing of protective fragment (Hc) of Clostridium **botulinum** neurotoxin serotype A (**BoNTa**)
AU Wang, Hui; Yin, Jun; Yuan, Bin; He, Jun; Wang, Zhongze
CS Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, 100071, Peop. Rep. China
SO Shengming Kexue Yanjiu (2001), 5(4), 325-328
CODEN: SKYAFL; ISSN: 1007-7847
PB Shengming Kexue Yanjiu Bianji Weiyuanhui
DT Journal
LA Chinese
AB The C-terminal half of the heavy chain gene of **botulinum** neurotoxin serotype A (BoNTaHc) was amplified and cloned into a sequencing plasmid pBluescript KS(II+). Cloned gene was sequenced and analyzed. A pair primers based on **BoNTa** complete sequence from Genbank and performed PCR amplification was designed. PCR product digested by restrictive endonuclease was ligated into pBluescript. Cloned gene was analyzed. A 1275 bp DNA fragment was amplified. Recombinant of pBlueBoNTaHc was constructed. Sequence anal. proved that the cloned gene was BoNTaHc gene. The successful cloning of BoNTaHc gene is the key to express in E. coli and further study on vaccine.

L13 ANSWER 6 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2000:53352 CAPLUS
DN 132:121456
TI **Botulinum** neurotoxin vaccine
IN Lee, John S.; Pushko, Peter; Smith, Jonathan F.; Parker, Michael; Dertzbaugh, Mark T.; Smith, Leonard
PA U.S. Medical Research Institute of Infectious Diseases, USA
SO PCT Int. Appl., 54 pp.
CODEN: PIXXD2

DT Patent
LA English
FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000002524	A2	20000120	WO 1999-US15570	19990709
	WO 2000002524	A3	20010531		
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	CA 2336587	AA	20000120	CA 1999-2336587	19990709
	AU 9954583	A1	20000201	AU 1999-54583	19990709
	AU 759461	B2	20030417		
	EP 1119626	A2	20010801	EP 1999-940801	19990709
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	US 2002034521	A1	20020321	US 1999-350756	19990709
	US 6495143	B2	20021217		
PRAI	US 1998-92416P	P	19980710		
	US 1999-133870P	P	19990512		
	WO 1999-US15570	W	19990709		

AB Using the nontoxic heavy chain fragment from **botulinum** neurotoxins serotype A-G, compns. and methods of use in inducing an immune response which is protective against intoxication with **botulinum** in subjects is described. Nontoxic fragments of these neurotoxins were inserted into the venezuelan equine encephalitis virus replicon vaccine vector system.

L13 ANSWER 7 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4
AN 1999:294914 BIOSIS
DN PREV199900294914
TI SNAP-25 requirement for dendritic growth of hippocampal neurons.
AU Grosse, Gisela; Grosse, Johannes; Tapp, Rosemarie; Kuchinke, Joerg; Gorsleben, Martin; Fetter, Ingmar; Hoehne-Zell, Barbara; Gratzl, Manfred (1); Bergmann, Mathias
CS (1) Anatomisches Institut der Technischen Universitaet Muenchen, Biedersteiner Str. 29, D-80802, Muenchen Germany
SO Journal of Neuroscience Research, (June 1, 1999) Vol. 56, No. 5, pp. 539-546.
ISSN: 0360-4012.
DT Article
LA English
SL English
AB Structure and dimension of the dendritic arbor are important determinants of information processing by the nerve cell, but mechanisms and molecules involved in dendritic growth are essentially unknown. We investigated early mechanisms of dendritic growth using mouse fetal hippocampal neurons in primary culture, which form processes during the first week in vitro. We detected a key component of regulated exocytosis, SNAP-25 (synaptosomal associated protein of 25 kDa), in axons and axonal terminals as well as in dendrites identified by the occurrence of the dendritic markers transferrin receptor and MAP2. Selective inactivation of SNAP-25 by **botulinum** neurotoxin A (**BoNTA**) resulted in inhibition of axonal growth and of vesicle recycling in axonal terminals. In addition, dendritic growth of hippocampal pyramidal and granule neurons was significantly inhibited by **BoNTA**. In contrast, cleavage of synaptobrevin by tetanus toxin had an effect on neither axonal nor

dendritic growth. Our observations indicate that SNAP-25, but not synaptobrevin, is involved in constitutive axonal growth and dendrite formation by hippocampal neurons.

L13 ANSWER 8 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5
AN 1998:518602 BIOSIS
DN PREV199800518602
TI Development of recombinant vaccines for **botulinum** neurotoxin.
AU Smith, Leonard A. (1)
CS (1) Dep. Immunol. Mol. Biol., Toxinol. Div., U.S. Army Med. Res. Inst. Infect. Dis., Fort Detrick, MD 21702-5011 USA
SO Toxicon, (Nov., 1998) Vol. 36, No. 11, pp. 1539-1548.
ISSN: 0041-0101.
DT Article
LA English
AB Synthetic genes encoding non-toxic, carboxyl-terminal regions (apprx50 kDa) of **botulinum** neurotoxin (BoNT) serotypes A and B (referred to as fragment C or HC) were constructed and cloned into the methylotropic yeast, Pichia pastoris. Genes specifying **BoNTA**(HC) and BoNTB(HC) were expressed as both intracellular and secreted products. Recombinants, expressed intracellularly, yielded products with the expected molecular weight as judged by SDS-PAGE and Western blot (immunoblot) analysis, while secreted products were larger due to glycosylation. Gene products were used to vaccinate mice and evaluated for their ability to elicit protective antibody titers in vivo. Mice given three intramuscular vaccinations with yeast supernatant containing glycosylated **BoNTA** (HC) were protected against an intraperitoneal challenge of 106 50% mouse lethal doses (MLD50) of serotype A neurotoxin, a result not duplicated by its BoNTB(HC) counterpart. Vaccinating mice with cytoplasmically produced **BoNTA**(HC) and BoNTB(HC) protected animals from a challenge of 106 MLD50 of serotype A and B toxins, respectively. Because of the glycosylation encountered with secreted BoNT(HC), our efforts focused on the production and purification of products from intracellular expression.

L13 ANSWER 9 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6
AN 1998:432879 BIOSIS
DN PREV199800432879
TI botR/A Is a positive regulator of **botulinum** neurotoxin and associated non-toxin protein genes in Clostridium **botulinum** A.
AU Marvaud, J. C.; Gilbert, M.; Inoue, K.; Fujinaga, Y.; Oguma, K.; Popoff, M. R. (1)
CS (1) Unite Toxines Microbiennes, Inst. Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15 France
SO Molecular Microbiology, (Aug., 1998) Vol. 29, No. 4, pp. 1009-1018.
ISSN: 0950-382X.
DT Article
LA English
AB The genes of the **botulinum** neurotoxin A (BoNT) complex are clustered in a locus consisting of two divergent polycistronic operons, one containing the non-toxic, non-haemagglutinin (NTNH) component and **bontA** genes, the other containing the haemagglutinin (HA) component genes. The two operons are separated by a gene (botR/A, previously called orf 21) encoding a 21 kDa protein. A recombinant Clostridium **botulinum** A strain that overexpresses botR/A was constructed by electroporating strain 62 with the vector pAT19 containing botR/A under the control of its own promoter. The transformed strain produced more BoNT/A and associated non-toxic proteins (ANTPs) and the corresponding mRNAs than the non-transformed strain. Partial inhibition of botR/A by antisense mRNA resulted in lower levels of BoNT/A, NTNH and HA70 and the levels of the corresponding mRNAs. Gel mobility shift assays and immunoprecipitations showed that BotR/A bound to the DNA promoter region

upstream from the two BoNT/A complex operons. These results show that botR/A activated transcription of the genes encoding BoNT/A and ANTPs in C. **botulinum** A by interacting directly with the region promoter, and that the homologous genes in C. **botulinum** B, C and D presumably have the same function.

L13 ANSWER 10 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1993:619459 CAPLUS
DN 119:219459
TI Proliferative T cell response to **botulinum** toxin type A in mice
AU Chan, Woon Ling; Sesardic, Dorothea; Shone, Clifford C.
CS Natl. Inst. Biol. Stand. Control, South Mimms/Potters Bar/Herts., EN6 3QG, UK
SO Botulinum Tetanus Neurotoxins [Proc. Int. Conf.] (1993), Meeting Date 1992, 337-9. Editor(s): Dasgupta, Bibhuti R. Publisher: Plenum, New York, N. Y.
CODEN: 59KIAW
DT Conference
LA English
AB Mice immunized with **botulinum** toxoid A were able to produce both in vitro proliferative T cell and antibody response to purified **BoNTA**/A fragments L and H. While there was no significant difference in the antibody titers of sera from mice given toxoid with or without alum as adjuvant, antibodies from gp2 sera showed higher binding and avidity to **BoNTA**/A fragment L than those from gp1. There was no such difference in the binding capacity of sera from both groups to toxoid. There was a much stronger in vitro antigen specific proliferative T cell response to all antigens tested. This suggests that the induction of good protective antibody response requires both T and B cells.

L13 ANSWER 11 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1993:643306 CAPLUS
DN 119:243306
TI Long-term effects of **botulinum** type A neurotoxin on the release of noradrenaline from PC12 cells
AU Shone, Clifford C.
CS Div. Biol., Cent. Appl. Microbiol. Res., Porton Down/Salisbury/Wilts., SP4 0JG, UK
SO Botulinum Tetanus Neurotoxins [Proc. Int. Conf.] (1993), Meeting Date 1992, 321-31. Editor(s): Dasgupta, Bibhuti R. Publisher: Plenum, New York, N. Y.
CODEN: 59KIAW
DT Conference
LA English
AB Clostridium **botulinum** type A neurotoxin (**BoNTA**) slowly inhibits the calcium-dependent release of noradrenaline from PC12 cells in a dose-dependent manner. The effects of **BoNTA** on PC12 cells are shown to persist for several days in in subsequent cell generations.

L13 ANSWER 12 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 7
AN 1992:478047 BIOSIS
DN BA94:109422
TI INHIBITION OF CALCIUM-DEPENDENT RELEASE OF NORADRENALINE FROM PC12 CELLS BY **BOTULINUM** TYPE-A NEUROTOXIN LONG-TERM EFFECTS OF THE NEUROTOXIN ON INTACT CELLS.
AU SHONE C C; MELLING J
CS DIV. BIOLOGICS, PHLS CAMR, PORTON DOWN, SALISBURY, WILTS SP4 OJG, ENGLAND.
SO EUR J BIOCHEM, (1992) 207 (3), 1009-1016.
CODEN: EJBCAI. ISSN: 0014-2956.
FS BA; OLD
LA English
AB Clostridium **botulinum** type-A neurotoxin (**BoNTA**)

inhibited the calcium-dependent release of noradrenaline from PC12 cells in a dose-dependent manner. Under conditions in which intact PC12 cells were incubated with **BoNTA** for 20 h at 37.degree. C, a neurotoxin concentration of approximately 0.12 .+-. 0.13 .mu.M was required to inhibit 50% of the calcium-dependent noradrenaline release. PC12 cells, differentiated in the presence of nerve growth factor for 14 days, showed a similar dose-dependent inhibition of noradrenaline release by **BoNTA** with unchanged sensitivity. No specific saturable binding of 125I-labeling **BoNTA** was observed to either differentiated or undifferentiated PC12 cells, suggesting a lack of high-affinity acceptors on the cell surface for the neurotoxin. It is proposed that **BoNTA** enters PC12 cells either by non-specific bindings to the cell membrane or via a low-concentration low-affinity acceptor molecule. A study of the long-term effects of **BoNTA** on noradrenaline release from PC12 cells showed that the neurotoxin remains active within the growing cells for several days. Noradrenaline release from PC12 cells exposed to **BoNTA** (0.3 .mu.M) for 24 h was reduced to less than 20% of control values over a subsequent 4-day period. After 8 days, release levels were significantly lower (60-65%) than control values, despite a more than 10-fold increase in the cell mass. Investigation of the subcellular distribution of **BoNTA** after incubation with PC12 cells for 96 h revealed the bulk of the toxin (94-98%) to be associated with the cell membrane fraction. Of this, 50-80% of the **BoNTA** was associated with the nuclear and cell debris fraction and 11-25% was recovered in the large-granule-vesicle fraction; the specific binding of the neurotoxin to these membrane fractions was found to be similar. Examination of the form of the cell-associated **BoNTA** after incubation for 96 h with PC12 cells revealed no evidence of any significant degradation of either neurotoxin subunit. This suggests that the neurotoxin adopts a relatively stable form within the cell. On SDS/PAGE under non-reducing conditions, no trace of protein bands corresponding to either of the **BoNTA** subunits were observed, suggesting that little or none of the neurotoxin subunits exists in a monomeric form within the cells. A considerable portion (50-70%) of the membrane-associated **BoNTA** was found to be present in the form of large disulphide-linked aggregates which, on SDS/PAGE in the presence of a thiol, dissociated into the bands corresponding to the neurotoxin heavy-chain and light-chain subunits. Examination of the neurotoxin aggregates associated with the dense-core-vesicle fraction showed them to contain a greater proportion of the light-chain subunit (29.3% .+-. 10.3) compared to the control toxin. The above observations are discussed in relation to the mode of action of **BoNTA**.

L13 ANSWER 13 OF 13 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1992:442347 CAPLUS
DN 117:42347
TI Intracellular form of **botulinum** type A neurotoxin after long-term incubation with PC12 cells
AU Shone, C. C.
CS Cent. Appl. Microbiol. Res., Porton Down/Salisbury/Wiltshire, SP4 0JG, UK
SO Zentralblatt fuer Bakteriologie, Supplement (1992), 23(Bact. Protein Toxins), 71-2
CODEN: ZBASE2; ISSN: 0941-018X
DT Journal
LA English
AB **Botulinum** type A neurotoxin (**BoNTA**) inhibits the calcium-mediated release of noradrenaline from PC12 cells in a dose-dependent manner. When PC12 cells are exposed to 0.3 .mu.M **BoNTA** for 24 h, washed and then cultured for a further 8 days, the levels of calcium-dependent noradrenaline release remain significantly lower than control values, suggesting that the neurotoxin is able to function for several days once inside the cell. In similar expts. performed with 125I-labeled **BoNTA**, the bulk of the neurotoxin

(.apprx.95%) was assocd. with the membrane fractions. Examn. of the membrane assocd. **BoNTA** on SDS-PAGE showed a significant portion (50-70%) to be in the form of large (>106 Daltons) aggregates linked by disulfide bonds.

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=> s botulinum or clostridial
L14      38094 BOTULINUM OR CLOSTRIDIAL

=> s l14 and snap-25
L15        891 L14 AND SNAP-25

=> s l15 and fluorophore
L16          4 L15 AND FLUOROPHORE

=> d bib 1-4

L16  ANSWER 1 OF 4  WPIDS  COPYRIGHT 2003 THOMSON DERWENT on STN
AN   2003-290198 [28]   WPIDS
DNC  C2003-075494
TI   Botulinum serotype A/E substrate useful for assaying protease
     activity of botulinum toxins, comprises donor
     fluorophore, acceptor and a clostridial toxin
     recognition sequence that includes a cleavage site.
DC   B04 D16
IN   AOKI, K R; FERNANDEZ-SALAS, E; STEWARD, L E
PA   (AOKI-I) AOKI K R; (FERN-I) FERNANDEZ-SALAS E; (STEW-I) STEWARD L E;
     (ALLR) ALLERGAN INC
CYC  100
PI   WO 2003020948 A2 20030313 (200328)* EN  168p
        RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
            MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
            KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
            RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
     US 2003143650 A1 20030731 (200354)
ADT  WO 2003020948 A2 WO 2002-US27145 20020822; US 2003143650 A1 US 2001-942024
     20010828
PRAI US 2001-942024   20010828

L16  ANSWER 2 OF 4  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
AN   2003-13448  BIOTECHDS
TI   Botulinum serotype A/E substrate useful for assaying protease
     activity of botulinum toxins, comprises donor
     fluorophore, acceptor and a clostridial toxin
     recognition sequence that includes a cleavage site;
          botulinum toxin protease activity analysis in bacterium,
        baculo virus, yeast lysate, food, beverage, feedstuff, soil, water,
        cosmetic and tissue sample
AU   STEWARD L E; FERNANDEZ-SALAS E; AOKI K R
PA   ALLERGAN INC
PI   WO 2003020948 13 Mar 2003
AI   WO 2002-US27145 22 Aug 2002
PRAI US 2001-942024 28 Aug 2001; US 2001-942024 28 Aug 2001
DT   Patent
LA   English
OS   WPI: 2003-290198 [28]

L16  ANSWER 3 OF 4  CAPLUS  COPYRIGHT 2003 ACS on STN
AN   2003:590711  CAPLUS
DN   139:129339
TI   Fluorophore-labeled peptides and FRET assays for
```

clostridial toxins
IN Steward, Lance E.; Fernandez-Salas, Ester; Aoki, Kei Roger
PA USA
SO U.S. Pat. Appl. Publ., 69 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2003143651	A1	20030731	US 2001-942098	20010828
PRAI US 2001-942098		20010828		

L16 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:202825 CAPLUS
DN 138:233337
TI FRET protease assays for **botulinum** serotype A/E toxins
IN Steward, Lance E.; Fernandez-Salas, Ester; Aoki, Kei Roger
PA Allergan, Inc., USA
SO PCT Int. Appl., 168 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2003020948	A2	20030313	WO 2002-US27145	20020822
WO 2003020948	A3	20030605		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003143650	A1	20030731	US 2001-942024	20010828
PRAI US 2001-942024	A	20010828		

=> s l14 and recogni? (5a) sequence
L17 19 L14 AND RECOGNI? (5A) SEQUENCE

=> dup rem l17
PROCESSING COMPLETED FOR L17
L18 9 DUP REM L17 (10 DUPLICATES REMOVED)

=> d bib ab 1-9

L18 ANSWER 1 OF 9 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 1
AN 2003-430498 [40] WPIDS
DNC C2003-113891
TI New nucleotide sequences and their encoded enzyme-deficient C3 **botulinum** proteins, useful for manufacturing an agent for diagnosis, prophylactic or therapeutic treatment of a damage of the central and/or peripheral nervous system.
DC B04 D16
IN AHNERT-HILGER, G; GROSSE, G; HOFMANN, F; JUST, I
PA (UYBE) UNIV BERLIN HUMBOLDT
CYC 101
PI WO 2003037920 A2 20030508 (200340)* EN 58p

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA
ZM ZW

ADT WO 2003037920 A2 WO 2002-EP12039 20021028

PRAI DE 2001-10154685 20011029

AB WO2003037920 A UPAB: 20030624

NOVELTY - A new polypeptide that promotes neurite outgrowth in mammals, is new.

DETAILED DESCRIPTION - A new polypeptide that promotes neurite outgrowth in mammals comprises:

(a) any one of 13 fully defined sequences of 14-211 amino acids (designated I-XIII) given in the specification;

(b) a sequence that has at least 40% homology with the sequences of I-XIII;

(c) a variant of the polypeptide of (a) or (b);

(d) a conservatively substituted variant of the polypeptide of (a), (b) or (c) comprising a substitution, deletion and/or insertion of one or more amino acids; or

(e) a functionally equivalent homologue of (a), (b), (c) or (d).

INDEPENDENT CLAIMS are included for the following:

(1) an isolated nucleotide sequence comprising:

(a) a nucleotide sequence encoding a polypeptide having neurite outgrowth activity comprising any one of the sequences of I-XIII;

(b) a nucleotide sequence that is complementary to the sequence of (a);

(c) a nucleotide sequences differing from the sequence of (a) or (b) in codon sequences due to the degeneracy of the genetic code, where the nucleotide sequence encodes a polypeptide having a biological activity indicated in (a);

(d) a nucleotide sequence that specifically hybridizes under stringent hybridization conditions to the sequence of (a), (b) or (c); or;

(e) a nucleotide sequence of (a), (b), (c) or (d) having a deletion, addition, substitution mutation, where the nucleotide sequence encodes a polypeptide having a biological activity indicated in (a);

(2) a vector comprising the isolated nucleotide sequence of (1);

(3) a host cell transfected with the vector;

(4) a recognition agent capable of **recognizing** the nucleotide **sequence** or the polypeptide;

(5) a pharmaceutical composition comprising the nucleotide **sequence**, the polypeptide, or the **recognition** agent, and one or more pharmaceutical adjuvant, excipient, carrier, buffer, diluent and/or customary pharmaceutical auxiliary;

(6) a neurite outgrowth-promoting apparatus comprising a bioabsorbable matrix and the pharmaceutical composition of (5);

(7) a kit for screening a molecule that binds the nucleotide **sequence**, the polypeptide or the **recognition** agent comprising the nucleotide **sequence**, the polypeptide, the **recognition** agent and/or the pharmaceutical composition;

(8) modulating neurite outgrowth of central and/or peripheral nervous system neurons in vitro or in vivo comprising contacting the neurons with the nucleotide **sequence**, the polypeptide, and/or the **recognition** agent, or the pharmaceutical composition; and

(9) identifying a receptor/molecule that binds the polypeptide comprising providing the polypeptide above, contacting the polypeptide with the candidate molecule, and detecting binding of the candidate molecule to the polypeptide.

ACTIVITY - Neuroprotective; Nootropic; Antiparkinsonian; Neuroleptic; Anticonvulsant; Ophthalmological; Tranquilizer; Vulnerary; Cerebroprotective. No biological data given.

MECHANISM OF ACTION - C3 Agonist.

USE - The nucleotide **sequence**, polypeptide, **recognition** agent and pharmaceutical composition are useful in promoting neural growth, for manufacturing an agent for diagnosis, prophylactic and/or therapeutic treatment of a damage of the central and/or peripheral nervous system, or for inducing an expansion and/or differentiation of stem cells. The method is also useful for modulating neurite outgrowth of central and/or peripheral nervous system neurons in vitro or in vivo, or for inducing neurite outgrowth in the central and/or peripheral nervous system of a patient with damage to the central and/or peripheral nervous system, such as infarction, traumatic injury, surgical lesion, a degenerative disorder of the central nervous system (e.g. Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, diffuse cerebral cortical atrophy, Lewy-body dementia, Pick's disease, mesolimbocortical dementia, thalamic degeneration, Huntington's chorea, cortical-striatal-spinal degeneration, cortical-basal-ganglionic degeneration, cerebrocerebellar degeneration, familial dementia with spastic paraparesis, polyglucosan body disease, Shy-Drager's syndrome, olivopontocerebellar atrophy, progressive supranuclear palsy, dystonia musculorum deformans, Hallervorden-Spatz's disease, Meige's syndrome, familial tremors, Gilles de la Tourette's syndrome, acanthocytic chorea, Friedreich's ataxia, Holmes' familial cortical cerebellar atrophy, Gerstmann-Straussler-Scheinker's disease, progressive spinal muscular atrophy, progressive balbar palsy, primary lateral sclerosis, hereditary muscular atrophy, spastic paraplegia, peroneal muscular atrophy, hypertrophic interstitial polyneuropathy, heredopathia atactica polyneuritiformis, optic neuropathy or ophthalmoplegia), or a damage in the spinal cord (all claimed).

Dwg.0/7

L18 ANSWER 2 OF 9 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 2
AN 2003-290198 [28] WPIDS
DNC C2003-075494
TI **Botulinum** serotype A/E substrate useful for assaying protease activity of **botulinum** toxins, comprises donor fluorophore, acceptor and a **clostridial** toxin **recognition sequence** that includes a cleavage site.
DC B04 D16
IN AOKI, K R; FERNANDEZ-SALAS, E; STEWARD, L E
PA (AOKI-I) AOKI K R; (FERN-I) FERNANDEZ-SALAS E; (STEW-I) STEWARD L E; (ALLR) ALLERGAN INC
CYC 100
PI WO 2003020948 A2 20030313 (200328)* EN 168p
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
US 2003143650 A1 20030731 (200354)
ADT WO 2003020948 A2 WO 2002-US27145 20020822; US 2003143650 A1 US 2001-942024 20010828
PRAI US 2001-942024 20010828
AB WO2003020948 A UPAB: 20030501

NOVELTY - A **botulinum** serotype A/E (BoNT/A/E) substrate, comprises a donor fluorophore, an acceptor having an absorbance spectrum overlapping the emission spectrum of donor fluorophore, and a BoNT A or BoNT/E **recognition sequence** comprising a cleavage site (the site intervenes between donor fluorophore and acceptor and under the appropriate conditions, resonance energy transfer is exhibited between the donor and acceptor).

USE - (I) is useful in assaying for the protease activity of any **clostridial** toxin, including **botulinum** toxins in a

sample including bacterial, baculoviral and yeast lysate, raw, cooked or processed foods, beverages, animal feed, soil samples, water samples, cosmetics, tissue samples, and food or beverage sample. (I) is useful to assay a sample from a human or animal, for e.g., exposed to a **clostridial** toxin, or having one or more symptoms of a **clostridial** toxin, to follow activity during production and purification of **clostridial** toxin, and to assay formulated **clostridial** toxin products, including pharmaceuticals and cosmetics.

ADVANTAGE - The **botulinum** toxin substrates are utilized in rapid and simple homogenous screening assays that do not require separation of cleaved product from uncleaved substrate and do not rely on toxicity to animals.

Dwg.0/7

L18 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:590711 CAPLUS
DN 139:129339
TI Fluorophore-labeled peptides and FRET assays for **clostridial** toxins
IN Steward, Lance E.; Fernandez-Salas, Ester; Aoki, Kei Roger
PA USA
SO U.S. Pat. Appl. Publ., 69 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003143651	A1	20030731	US 2001-942098	20010828
PRAI US 2001-942098		20010828		

AB The present invention provides **clostridial** toxin substrates useful in assaying for the protease activity of any **clostridial** toxin, including **botulinum** toxins of all serotypes as well as tetanus toxins. A **clostridial** toxin substrate of the invention contains a donor fluorophore; an acceptor having an absorbance spectrum overlapping the emission spectrum of the donor fluorophore; and a **clostridial** toxin **recognition sequence** that includes a cleavage site, where the cleavage site intervenes between the donor fluorophore and the acceptor and where, under the appropriate conditions, resonance energy transfer is exhibited between the donor fluorophore and the acceptor.

L18 ANSWER 4 OF 9 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 3
AN 2003-093128 [08] WPIDS
DNC C2003-023374
TI Identifying the target of a compound which inhibits cellular proliferation, comprises contacting a culture of strains that overexpress or underexpress a gene product with the above compound, and identifying the gene product.
DC B04 D16
IN BOONE, C; BUSSEY, H; CARR, G J; FOULKES, G J; HASELBECK, R; JIANG, B; OHLSEN, K L; ROEMER, T; TRAWICK, J D; WALL, D; XU, H H; YAMAMOTO, R T; ZAMUDIO, C; ZYSKIND, J W
PA (ELIT-N) ELITRA PHARM INC
CYC 100
PI WO 2002086097 A2 20021031 (200308)* EN 640p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM

ZW
ADT WO 2002086097 A2 WO 2002-US3987 20020208
PRAI US 2001-267636P 20010209
AB WO 200286097 A UPAB: 20030204
NOVELTY - Identifying gene products on which compounds inhibiting proliferation of an organism act, comprising obtaining a culture of strains overexpressing a different product for proliferation of the organism, contacting the culture with a compound to inhibit proliferation of strains that do not overexpress the product, and identifying the product overexpressed in a strain that proliferated more rapidly, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) profiling a compound's activity;
(2) a culture comprising several strains, where each strain overexpresses a different gene product that is essential for proliferation of the organism;
(3) determining the extent to which each of the strains are present in a culture or a collection of strains; and
(4) identifying the target of the compound which inhibits the proliferation of an organism.
USE - The method is useful in identifying the target of a compound which reduces the activity or level of gene products required for cellular proliferation. The method may also be used for identifying the therapeutic compounds that act on the novel targets.
Dwg.0/19

L18 ANSWER 5 OF 9 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 4
AN 2003-029926 [02] WPIDS
CR 2001-611495 [70]; 2002-575374 [61]; 2003-479541 [45]
DNC C2003-006812
TI New antisense nucleic acids, useful for identifying proteins or screening for homologous nucleic acids required for cellular proliferation to isolate candidate molecules for rational drug discovery programs.
DC B04 D16
IN CARR, G J; FORSYTH, R A; HASELBECK, R; MALONE, C; OHLSEN, K L; TRAWICK, J D; WALL, D; WANG, L; XU, H H; YAMAMOTO, R; ZAMUDIO, C; ZYSKIND, J W
PA (ELIT-N) ELITRA PHARM INC
CYC 100
PI WO 2002077183 A2 20021003 (200302)* EN 863p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW
ADT WO 2002077183 A2 WO 2002-US9107 20020321
PRAI US 2002-362699P 20020306; US 2001-815242 20010321; US 2001-948993 20010906; US 2001-342923P 20011025; US 2002-72851 20020208
AB WO 200277183 A UPAB: 20030716
NOVELTY - Isolated nucleic acid comprising any one of the 6213 sequences given in the specification where expression of the nucleic acid inhibits proliferation of a cell, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:
(1) a vector comprising a promoter operably linked to the nucleic acid encoding a polypeptide whose expression is inhibited by the antisense nucleic acid;
(2) a host cell containing the vector;
(3) an isolated polypeptide or its fragment whose expression is inhibited by the antisense nucleic acid;
(4) an antibody capable of specifically binding the polypeptide;
(5) producing the polypeptide;
(6) inhibiting cellular proliferation or the activity of a gene in an

operon required for proliferation;

(7) identifying a compound that influences the activity of the gene product or that has an activity against a biological pathway required for proliferation, or that inhibits cellular proliferation;

(8) identifying a compound or nucleic acid that reduces the activity or level of the gene product required for proliferation or that interacts with the gene or gene product to inhibit cellular proliferation;

(9) a composition comprising the antisense nucleic acid or its proliferation-inhibiting portion in a carrier;

(10) identifying a gene required for cellular proliferation or the biological pathway in which a proliferation-required gene or its gene product lies or a gene on which the test compound that inhibits proliferation of an organism acts;

(11) manufacturing an antibiotic;

(12) profiling a compound's activity;

(13) a culture comprising strains in which the gene product is overexpressed or underexpressed;

(14) determining the extent to which each of the strains is present in a culture or collection of strains; or

(15) identifying the target of a compound that inhibits the proliferation of an organism.

USE - The antisense nucleic acids are useful for identifying proteins or screening for homologous nucleic acids required for cellular proliferation to isolate candidate molecules for rational drug discovery programs, or for screening homologous nucleic acids required for proliferation in cells other than S. aureus, S. typhimurium, K. pneumoniae or P. aeruginosa.

Dwg.0/18

L18 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2002:186350 BIOSIS
DN PREV200200186350
TI Inhibition of Rho mediated signaling pathway promotes proplatelet formation through the activation of NF-E2.
AU Yamada, Wakako (1); Morita, Haruhiko (1); Murakami, Yasunobu (1); Nakamura, Sawako (1); Motohashi, Hozumi; Yamamoto, Masayuki; Kato, Takashi (1)
CS (1) Pharmaceutical Research Laboratory, Kirin Brewery Co., Ltd., Takasaki, Gunma Japan
SO Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 287a. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DT Conference
LA English
AB Terminally differentiated megakaryocytes shed hundreds of platelets through the intermediate structure called "proplatelet". The pricise mechanisms of platelet production, however, has not been elucidated. Although the proliferation and differentiation of megakaryocytes is mainly regulated by TPO, proplatlet formation (PPF) itself seems to be regulated independently for several reasons as follows: First, as shown in previous studies, mice lacking TPO or c-mpl gene showed 15% of normal mice platelets. (ref. Blood 90:3423, Science 265:1445) Secondary, excess amount of TPO suppressed PPF in vitro. The transcription factor NF-E2, composed of two basic-leucine zipper subunits of p45 and small Maf proteins, exhibited a sequence specific transcriptional activator. Mice lacking p45 show profound thrombocytepenia associated with increased megakaryocytes and defect in PPF, implicated NF-E2 as an essential regulator of terminal megakaryocyte defferntiation and platelet. We developed a cell-based high throughput reporter gene assay system to screen small organic compounds which stimulate PPF. Human megakaryocytic cell line Meg01 cells were stably transfected with luciferase gene reporter construct under the

control of NF-E2 **recognition sequence**. Using this reporter assay system, we found that H-9 (N-(2-Aminoethyl)isoquinoline-5-sulfonamide hydrochloride), a PKA and PKC inhibitor, and fasudil (hexahydro-1-(5-isoquinolinesulfonyl)-1H-1,4-diazepine hydrochloride), a Rho kinase inhibitor, structually related isoquinolinesulfonamide delivatives, shown markedly elevated luciferase gene expression. To investigate whether they are able to promote PPF, rat primary megakaryocytes were cultured, and consequently, H-9 and fasudil promote PPF. We examined the effect of **botulinum** C3 exoenzyme, a Rho kinase inactivating enzyme, and it also shown PPF promoting effect as expected. We then introduced dominant-negative Rho kinase coding genes into primary megakaryocytes and they developed PPF. These results demonstrate that morphological change of PPF megakaryocyte is accompanied by inhibition of Rho kinase mediated signal transduction pathway. Taken together, these compounds provide important information of celluar events in PPF and platelet production.

L18 ANSWER 7 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5
AN 1998:503771 BIOSIS
DN PREV199800503771
TI On the action of **botulinum** neurotoxins A and E at cholinergic terminals.
AU Washbourne, Philip; Pellizzari, Rossella; Rossetto, Ornella; Bortoletto, Nicola; Tugnoli, Valeria; De Grandis, Domenico; Eleopra, Roberto; Montecucco, Cesare (1)
CS (1) Centro CNR Biomembrane, Univ. Padova, via G. Colombo 3, 35100 Padova Italy
SO Journal of Physiology Paris, (April, 1998) Vol. 92, No. 2, pp. 135-139. ISSN: 0928-4257.
DT Article
LA English
SL English; French
AB **Botulinum** neurotoxins type A and E (BoNT/A and /E) are metalloproteases with a unique specificity for SNAP-25 (synaptosomal-associated protein of 25 kDa), an essential protein component of the neuroexocytotic machinery. It was proposed that this specificity is based on the **recognition** of a nine-residue **sequence**, termed SNARE motif, which is common to the other two SNARE proteins: VAMP (vesicle-associated membrane protein) and syntaxin, the only known substrates of the other six **clostridial** neurotoxins. Here we report on recent studies which provide evidence for the involvement of the SNARE motif present in SNAP-25 in its interaction with BoNT/A and /E by following the kinetics of proteolysis of SNAP-25 mutants deleted of SNARE motifs. We show that a single copy of the motif is sufficient for BoNT/A and /E to recognise SNAP-25. While the copy of the motif proximal to the cleavage site is clearly involved in recognition, in its absence, other more distant copies of the motif are able to support proteolysis. We also report on studies of poisoning human neuromuscular junctions with either BoNT/A or BoNT/E and describe the unexpected finding that the time of recovery of function after poisoning is much shorter in the case of type E with respect to type A intoxication. These data are discussed in terms of the different sites of action of the two toxins within SNAP-25.

L18 ANSWER 8 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 6
AN 1998:42481 BIOSIS
DN PREV199800042481
TI **Botulinum** neurotoxin types A and E require the SNARE motif in SNAP-25 for proteolysis.
AU Washbourne, Philip; Pellizzari, Rossella; Baldini, Giulia; Wilson, Michael C.; Montecucco, Cesare (1)

CS (1) Centro C.N.R. Biomembrane, Dipartimento Scienze Biomediche, Univ. Padova, Via Colombo 3, 35100 Padua Italy
SO FEBS Letters, (Nov. 24, 1997) Vol. 418, No. 1-2, pp. 1-5.
ISSN: 0014-5793.
DT Article
LA English
AB **Botulinum** neurotoxins type A and E (BoNT/A and BoNT/E) are metalloproteases with a unique specificity for SNAP-25 (synaptosome-associated protein of 25 kDa), an essential protein component of the neuroexocytotic machinery. It has been suggested that this specificity is directed through the **recognition** of a nine residue **sequence**, termed SNARE motif, that is common to the other two SNARE proteins: VAMP (vesicle-associated membrane protein) and syntaxin, the only known substrates of the other six **clostridial** neurotoxins. Here we analyse the involvement of the four copies of the SNARE motif present in SNAP-25 in its interaction with BoNT/A and BoNT/E by following the kinetics of proteolysis of SNAP-25 mutants deleted of SNARE motifs. We show that a single copy of the motif is sufficient for BoNT/A and BoNT/E to recognize SNAP-25. While the copy of the motif proximal to the cleavage site is clearly involved in recognition, in its absence, other more distant copies of the motif are able to support proteolysis. Also, a non-neuronal isoform of SNAP-25, Syndet, is shown to be sensitive to BoNT/E, but not BoNT/A, whilst the SNAP-25 isoforms from Torpedo marmorata and Drosophila melanogaster were demonstrated not to be substrates of these metalloproteases.

L18 ANSWER 9 OF 9 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
AN 1989-03568 BIOTECHDS
TI Restriction endonucleases in Clostridium pasteurianum ATCC 6013 and C. thermohydrosulfuricum DSM 568;
Clostridium thermohydrosulfuricum restriction endonuclease isolation
AU Richards D F; Linnett P E; Oultram J D; *Young M
CS Shell
LO Department of Botany and Microbiology, University College of Wales, Aberystwyth, Dyfed SY23 3DA, UK.
SO J.Gen.Microbiol.; (1988) 134, Pt.12, 3151-57
CODEN: JGMIAN
DT Journal
LA English
AB In a survey of 11 strains of Clostridium, type II restriction endonucleases were detected in 2 organisms; Clostridium pasteurianum ATCC 6013 and Clostridium thermohydrosulfuricum DSM 568, using phage lambda DNA as substrate. C. acetobutylicum was grown anaerobically at 37 deg in TYG medium (30 g/l Tryptone, 20 g/l yeast extract, 5 g/l glucose, 1 g/l Na-mercaptoacetate). C. thermohydrosulfuricum was grown anaerobically at 65 deg in reinforced **clostridial** medium. The enzyme from C. pasteurianum was denoted CpaAI, and was most active at pH 7.4 in the presence of 15 mM NaCl. Substrates were completely degraded at NaCl concentrations above 30 mM. CpaAI is an isoschizomer of ThaI. The enzyme CtyI from C. thermohydrosulfuricum has a specificity similar to that of MboI. Activity of CtyI was greatest at 65-70 deg, and was negligible below 40 deg. At suboptimal temp. digestion was best at pH 7.4-7.9. CtyI was active throughout the entire range of salt concentrations tested, i.e. 0-150 mM NaCl. Cleavage of the **recognition sequence** is prevented if adenine in the sequence is methylated. (36 ref)

=> d his

(FILE 'HOME' ENTERED AT 13:34:42 ON 28 SEP 2003)

FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS,

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     LIFESCI, CAPLUS' ENTERED AT 13:34:59 ON 28 SEP 2003
                  E STEWARD LANCE E/AU
L1             15 S E3-E4
                  E FERNANDEZ-SALAS ESTER/AU
                  E FERNANDEZ SALAS ESTER/AU
L2             65 S E1-E5
                  E AOKI KEI ROGER/AU
L3             25 S E2-E3
                  E AOKI K R/AU
L4            125 S E3-E4
L5            211 S L1-L4
L6              3 S L5 AND BOTULINUM (10A) FLUOROPHORE
L7             21 S L5 AND CLOSTRIDIAL
L8             12 DUP REM L7 (9 DUPLICATES REMOVED)
L9             55 S BONTA
L10             0 S L9 AND CLOSTRIDIAL
L11            37 S L9 AND BOTULINUM
L12             0 S L11 AND FLUOROPHORE
L13            13 DUP REM L11 (24 DUPLICATES REMOVED)
L14         38094 S BOTULINUM OR CLOSTRIDIAL
L15           891 S L14 AND SNAP-25
L16             4 S L15 AND FLUOROPHORE
L17            19 S L14 AND RECOGNI? (5A) SEQUENCE
L18             9 DUP REM L17 (10 DUPLICATES REMOVED)

=> s l15 and (edans or qsy7 or fluorescein or alexafluoro488)
L19             4 L15 AND (EDANS OR QSY7 OR FLUORESCEIN OR ALEXAFLUORO488)

=> dup rem l19
PROCESSING COMPLETED FOR L19
L20             2 DUP REM L19 (2 DUPLICATES REMOVED)

=> d bib ab 1-2

L20  ANSWER 1 OF 2  WPIDS  COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 1
AN   2003-290198 [28]   WPIDS
DNC  C2003-075494
TI   Botulinum serotype A/E substrate useful for assaying protease
     activity of botulinum toxins, comprises donor fluorophore,
     acceptor and a clostridial toxin recognition sequence that
     includes a cleavage site.
DC   B04 D16
IN   AOKI, K R; FERNANDEZ-SALAS, E; STEWARD, L E
PA   (AOKI-I) AOKI K R; (FERN-I) FERNANDEZ-SALAS E; (STEW-I) STEWARD L E;
     (ALLR) ALLERGAN INC
CYC  100
PI   WO 2003020948 A2 20030313 (200328)* EN  168p
        RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
            MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
            KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
            RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
     US 2003143650 A1 20030731 (200354)
ADT  WO 2003020948 A2 WO 2002-US27145 20020822; US 2003143650 A1 US 2001-942024
     20010828
PRAI US 2001-942024   20010828
AB   WO2003020948 A UPAB: 20030501
     NOVELTY - A botulinum serotype A/E (BoNT/A/E) substrate,
     comprises a donor fluorophore, an acceptor having an absorbance spectrum
     overlapping the emission spectrum of donor fluorophore, and a BoNT A or
     BoNT/E recognition sequence comprising a cleavage site (the site
     intervenes between donor fluorophore and acceptor and under the
```

appropriate conditions, resonance energy transfer is exhibited between the donor and acceptor).

USE - (I) is useful in assaying for the protease activity of any **clostridial** toxin, including **botulinum** toxins in a sample including bacterial, baculoviral and yeast lysate, raw, cooked or processed foods, beverages, animal feed, soil samples, water samples, cosmetics, tissue samples, and food or beverage sample. (I) is useful to assay a sample from a human or animal, for e.g., exposed to a **clostridial** toxin, or having one or more symptoms of a **clostridial** toxin, to follow activity during production and purification of **clostridial** toxin, and to assay formulated **clostridial** toxin products, including pharmaceuticals and cosmetics.

ADVANTAGE - The **botulinum** toxin substrates are utilized in rapid and simple homogenous screening assays that do not require separation of cleaved product from uncleaved substrate and do not rely on toxicity to animals.

Dwg.0/7

L20 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:590711 CAPLUS
DN 139:129339
TI Fluorophore-labeled peptides and FRET assays for **clostridial** toxins
IN Steward, Lance E.; Fernandez-Salas, Ester; Aoki, Kei Roger
PA USA
SO U.S. Pat. Appl. Publ., 69 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2003143651	A1	20030731	US 2001-942098	20010828
PRAI US 2001-942098		20010828		

AB The present invention provides **clostridial** toxin substrates useful in assaying for the protease activity of any **clostridial** toxin, including **botulinum** toxins of all serotypes as well as tetanus toxins. A **clostridial** toxin substrate of the invention contains a donor fluorophore; an acceptor having an absorbance spectrum overlapping the emission spectrum of the donor fluorophore; and a **clostridial** toxin recognition sequence that includes a cleavage site, where the cleavage site intervenes between the donor fluorophore and the acceptor and where, under the appropriate conditions, resonance energy transfer is exhibited between the donor fluorophore and the acceptor.

=> d his

(FILE 'HOME' ENTERED AT 13:34:42 ON 28 SEP 2003)

FILE 'BIOSIS, MEDLINE, AGRICOLA, EMBASE, CABA, WPIDS, JAPIO, BIOTECHDS, LIFESCI, CAPLUS' ENTERED AT 13:34:59 ON 28 SEP 2003

		E	STEWARD LANCE E/AU
L1	15	S	E3-E4
		E	FERNANDEZ-SALAS ESTER/AU
		E	FERNANDEZ SALAS ESTER/AU
L2	65	S	E1-E5
		E	AOKI KEI ROGER/AU
L3	25	S	E2-E3
		E	AOKI K R/AU
L4	125	S	E3-E4
L5	211	S	L1-L4

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L6            3 S L5 AND BOTULINUM (10A) FLUOROPHORE
L7           21 S L5 AND CLOSTRIDIAL
L8           12 DUP REM L7 (9 DUPLICATES REMOVED)
L9           55 S BONTA
L10           0 S L9 AND CLOSTRIDIAL
L11          37 S L9 AND BOTULINUM
L12           0 S L11 AND FLUOROPHORE
L13          13 DUP REM L11 (24 DUPLICATES REMOVED)
L14       38094 S BOTULINUM OR CLOSTRIDIAL
L15         891 S L14 AND SNAP-25
L16           4 S L15 AND FLUOROPHORE
L17          19 S L14 AND RECOGNI? (5A) SEQUENCE
L18           9 DUP REM L17 (10 DUPLICATES REMOVED)
L19           4 S L15 AND (EDANS OR QSY7 OR FLUORESCEIN OR ALEXAFLUORO488)
L20           2 DUP REM L19 (2 DUPLICATES REMOVED)

=> s l14 and clostridial (5a) toxin (5a) substrate
L21           5 L14 AND CLOSTRIDIAL (5A) TOXIN (5A) SUBSTRATE

=> dup rem l21
PROCESSING COMPLETED FOR L21
L22           4 DUP REM L21 (1 DUPLICATE REMOVED)

=> d bib ab 1-4

L22   ANSWER 1 OF 4  BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
AN    2003-13448  BIOTECHDS
TI    Botulinum serotype A/E substrate useful for assaying protease
      activity of botulinum toxins, comprises donor fluorophore,
      acceptor and a clostridial toxin recognition sequence that
      includes a cleavage site;
           botulinum toxin protease activity analysis in bacterium,
         baculo virus, yeast lysate, food, beverage, feedstuff, soil, water,
         cosmetic and tissue sample
AU    STEWARD L E; FERNANDEZ-SALAS E; AOKI K R
PA    ALLERGAN INC
PI    WO 2003020948 13 Mar 2003
AI    WO 2002-US27145 22 Aug 2002
PRAI  US 2001-942024 28 Aug 2001; US 2001-942024 28 Aug 2001
DT    Patent
LA    English
OS    WPI: 2003-290198 [28]
AB    DERWENT ABSTRACT:
      NOVELTY - A botulinum serotype A/E (BoNT/A/E) substrate,
      comprises a donor fluorophore, an acceptor having an absorbance spectrum
      overlapping the emission spectrum of donor fluorophore, and a BoNT A or
      BoNT/E recognition sequence comprising a cleavage site (the site
      intervenes between donor fluorophore and acceptor and under the
      appropriate conditions, resonance energy transfer is exhibited between
      the donor and acceptor).
           WIDER DISCLOSURE - Also disclosed are: (1) BoNT/B, BoNT/C1, BoNT/D,
      BoNT/F, and BoNT/G substrates and their use for determining protease
      activity; (2) tetanus toxin (TeNT) substrate; (3)
      composite clostridial toxin substrate; and
      (4) kit for determining clostridial toxin protease
      activity in a sample.
           BIOTECHNOLOGY - Preferred Substrate: (I) is a BoNT/A substrate and
      comprises a BoNT/A recognition sequence comprising a cleavage site, or is
      a BoNT/E substrate and comprises a BoNT/E recognition sequence comprising
      a cleavage site. (I) comprises at least 6 consecutive residues of
      SNAP-25, comprising Gln-Arg (Gln(197)-Arg(198)) or Arg-Ile
      (Arg(180)-Ile(181)), or its peptidomimetic. (I) can be cleaved with an
      activity of at least 1, 20, 50, 100 or 150 nmol/minute/mg toxin. The
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acceptor is an acceptor fluorophore having a fluorescent lifetime of at least 1 microsecond. The acceptor is non-fluorescent. The donor fluorophore is fluorescein, Alexa Fluor (RTM), DABCYL, BODIPY. The acceptor is tetramethylrhodamine, EDANS, QSY (RTM) 7. The peptide or peptidomimetic has at most 20-100 residues. The donor fluorophore and acceptor fluorophore are separated by at most 15 residues, preferably 6 residues.

USE - (I) is useful in assaying for the protease activity of any **clostridial** toxin, including **botulinum** toxins in a sample including bacterial, baculoviral and yeast lysate, raw, cooked or processed foods, beverages, animal feed, soil samples, water samples, cosmetics, tissue samples, and food or beverage sample. (I) is useful to assay a sample from a human or animal, for e.g., exposed to a **clostridial** toxin, or having one or more symptoms of a **clostridial** toxin, to follow activity during production and purification of **clostridial** toxin, and to assay formulated **clostridial** toxin products, including pharmaceuticals and cosmetics.

ADVANTAGE - The **botulinum** toxin substrates are utilized in rapid and simple homogenous screening assays that do not require separation of cleaved product from uncleaved substrate and do not rely on toxicity to animals.

EXAMPLE - The fluorescent resonance energy transfer (FRET) substrate (A3) was synthesized by Alpha Diagnostics. X1-Asp-Ser-Asn-Lys-Thr-Arg-Ile-Asp-Glu-Ala-Asn- Gln-Arg-Ala-Thr-Lys-Met-Leu-Z2-NH2 (A3) This substrate contained a recognition sequence for BoNT/A flanked by a fluorescein-modified lysine residue (X1) and a tetramethylrhodamine-modified lysine residue (Z2) followed by a carboxy-terminal amide. Following proteolysis of **botulinum** toxin serotype A, the cleavage products (A4) were produced. X1-Asp-Ser-Asn-Lys-Thr-Arg-Ile-Asp-Glu-Ala-Asn-Gln and Arg-Ala-Thr-Lys-Met-Leu-Z2-NH2 (A4) Purified BoNT/A light chain (LC/A) or cellular extract containing LC/A was diluted in assay buffer. Dichain BoNT/A was incubated with 10 mM dithiothreitol (DTT) for about 30 minutes prior to analysis. Reactions contained various concentrations of LC/A, dichain toxin or formulated BOTOX (RTM) product, from 0.1 ng to 10 microg. Toxin was assayed. FRET substrate was added to a final concentration of 10 microM in a final volume of 100 microl assay buffer. The reaction is incubated at 37degreesC for 30 minutes, and was subsequently terminated by addition of 50 microl 2 M H2SO4. Fluorescence was measured with lambda(ex)= 488, lambda(Em) = 520 nm and lambda(em) = 585 nm. A reduction of at least 5% in the lambda(em) = 585 nm was indicative of BoNT/A protease activity. An increase of about 5% in the lambda(em) = 520 nm also was indicative of BoNT/A protease activity of the dichain or light chain **botulinum** toxin. These results demonstrated that **botulinum** toxin proteolytic activity can be assayed with an intramolecularly quenched FRET substrate.(168 pages)

L22 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:590711 CAPLUS
DN 139:129339
TI Fluorophore-labeled peptides and FRET assays for **clostridial** toxins
IN Steward, Lance E.; Fernandez-Salas, Ester; Aoki, Kei Roger
PA USA
SO U.S. Pat. Appl. Publ., 69 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2003143651	A1	20030731	US 2001-942098	20010828
PRAI	US 2001-942098		20010828		

AB The present invention provides **clostridial** toxin substrates useful in assaying for the protease activity of any **clostridial** toxin, including **botulinum** toxins of all serotypes as well as tetanus toxins. A **clostridial toxin substrate** of the invention contains a donor fluorophore; an acceptor having an absorbance spectrum overlapping the emission spectrum of the donor fluorophore; and a **clostridial** toxin recognition sequence that includes a cleavage site, where the cleavage site intervenes between the donor fluorophore and the acceptor and where, under the appropriate conditions, resonance energy transfer is exhibited between the donor fluorophore and the acceptor.

L22 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
AN 1990-01805 BIOTECHDS
TI Recent advances in the genetics of the clostridia; Clostridium sp. transformation, vector development, gene cloning, structure and expression, etc.; review
AU Young M; Minton N P; Staudenbauer W L
LO Department of Biological Sciences, University College of Wales, Aberystwyth, Dyfed SY23 3DA, UK.
SO FEMS Microbiol.Rev.; (1989) 63, 4, 310-26
CODEN: FMREE4
DT Journal
LA English
AB Clostridium sp. genetics are reviewed with respect to: (a) transformation (natural transformation, protoplast transformation, whole cell electroporation); (b) transduction; (c) conjugation (indigenous conjugative plasmid, streptococcal conjugative plasmid, plasmid mobilization from Escherichia coli mediated by conjugative IncP-group plasmid); (d) transposons (indigenous transposon, streptococcal transposon); (e) vector development (indigenous **clostridial** plasmids, vectors for use in Clostridium perfringens, Clostridium acetobutylicum, vector stability); (f) cloning of **clostridial** genes (**toxin** genes, genes concerned with **substrate** utilization and fermentative metabolism, etc.); (g) gene structure and expression (general considerations, transcription signals, ribosome binding sites, codon usage); and (h) future prospects. The essential elements of vector and gene transfer technology are established and may be applied to problems of fundamental and/or biotechnological interest e.g. molecular basis of oxygen sensitivity, toxoid production, implications of very biased codon usage for heterologous gene expression in clostridia, etc. (196 ref)

L22 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1977:157234 BIOSIS
DN BA63:52098
TI AVIAN BOTULISM EPIZOOTIOLOGY ON SEWAGE OXIDATION PONDS IN UTAH USA.
AU MOULTON D W; JENSEN W I; LOW J B
SO J WILDL MANAGE, (1976) 40 (4), 735-742.
CODEN: JWMAA9. ISSN: 0022-541X.
FS BA; OLD
LA Unavailable
AB In the microenvironment concept of avian botulism epizootiology, it is hypothesized that invertebrate carasses may serve as a substrate for toxin production by Clostridium **botulinum** type C and as a vehicle for toxin transmission to water birds. That hypothesis was field-tested by attempting to induce botulism in wing-clipped mallard ducks (Anas platyrhynchos) on sewage oxidation ponds in Utah, USA. The experimental ponds were inoculated with C. **botulinum** spores in June 1974. Aquatic insect populations were monitored throughout the summer. Rotenone was used in August to kill insects in 2 ponds (1 served as control), thereby providing potential **substrate** for **clostridial** growth and **toxin** production. Botulism was not detected among the

birds even though they routinely ingested invertebrate carcasses. Samples of dead invertebrates contained no **botulinum** toxin. The microenvironment concept, as it now stands, cannot always be a sufficient explanation of how type C botulism epizootics are initiated in nature. Other microbes may inhibit the growth of **clostridial** cells or destroy **botulinum** toxin.

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=> s snap 25
L23        3726 SNAP 25

=> s l23 and botulinum
L24         841 L23 AND BOTULINUM

=> s l24 and toxin (5a) substrate
L25          24 L24 AND TOXIN (5A) SUBSTRATE

=> dup rem l25
PROCESSING COMPLETED FOR L25
L26          11 DUP REM L25 (13 DUPLICATES REMOVED)

=> d bib ab 1-22

L26  ANSWER 1 OF 11  WPIDS  COPYRIGHT 2003 THOMSON DERWENT on STN DUPLICATE 1
AN   2003-290198 [28]   WPIDS
DNC  C2003-075494
TI   Botulinum serotype A/E substrate useful for assaying protease
     activity of botulinum toxins, comprises donor fluorophore,
     acceptor and a clostridial toxin recognition sequence that includes a
     cleavage site.
DC   B04 D16
IN   AOKI, K R; FERNANDEZ-SALAS, E; STEWARD, L E
PA   (AOKI-I) AOKI K R; (FERN-I) FERNANDEZ-SALAS E; (STEW-I) STEWARD L E;
     (ALLR) ALLERGAN INC
CYC  100
PI   WO 2003020948 A2 20030313 (200328)* EN  168p
        RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU
            MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
         W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK
            DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR
            KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT
            RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VN YU ZA ZM ZW
     US 2003143650 A1 20030731 (200354)
ADT  WO 2003020948 A2 WO 2002-US27145 20020822; US 2003143650 A1 US 2001-942024
     20010828
PRAI US 2001-942024   20010828
AB   WO2003020948 A UPAB: 20030501
     NOVELTY - A botulinum serotype A/E (BoNT/A/E) substrate,
     comprises a donor fluorophore, an acceptor having an absorbance spectrum
     overlapping the emission spectrum of donor fluorophore, and a BoNT A or
     BoNT/E recognition sequence comprising a cleavage site (the site
     intervenes between donor fluorophore and acceptor and under the
     appropriate conditions, resonance energy transfer is exhibited between the
     donor and acceptor).
          USE - (I) is useful in assaying for the protease activity of any
     clostridial toxin, including botulinum toxins in a sample
     including bacterial, baculoviral and yeast lysate, raw, cooked or
     processed foods, beverages, animal feed, soil samples, water samples,
     cosmetics, tissue samples, and food or beverage sample. (I) is useful to
     assay a sample from a human or animal, for e.g., exposed to a clostridial
     toxin, or having one or more symptoms of a clostridial toxin, to follow
     activity during production and purification of clostridial toxin, and to
     assay formulated clostridial toxin products, including pharmaceuticals and
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cosmetics.

ADVANTAGE - The **botulinum** toxin substrates are utilized in rapid and simple homogenous screening assays that do not require separation of cleaved product from uncleaved substrate and do not rely on toxicity to animals.

Dwg.0/7

L26 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:590711 CAPLUS
DN 139:129339
TI Fluorophore-labeled peptides and FRET assays for clostridial toxins
IN Steward, Lance E.; Fernandez-Salas, Ester; Aoki, Kei Roger
PA USA
SO U.S. Pat. Appl. Publ., 69 pp.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 2003143651	A1	20030731	US 2001-942098	20010828
PRAI US 2001-942098		20010828		

AB The present invention provides clostridial toxin substrates useful in assaying for the protease activity of any clostridial toxin, including **botulinum** toxins of all serotypes as well as tetanus toxins. A clostridial **toxin substrate** of the invention contains a donor fluorophore; an acceptor having an absorbance spectrum overlapping the emission spectrum of the donor fluorophore; and a clostridial toxin recognition sequence that includes a cleavage site, where the cleavage site intervenes between the donor fluorophore and the acceptor and where, under the appropriate conditions, resonance energy transfer is exhibited between the donor fluorophore and the acceptor.

L26 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:17804 CAPLUS
DN 138:68270
TI FRET substrate peptides and assays for detecting and measuring proteolytic activity of **botulinum** type A neurotoxin
IN Shine, Nancy Rose; Crawford, Karen Renee; Eaton, Linda Jo Ann
PA USA
SO U.S., 14 pp.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 6504006	B1	20030107	US 2001-976535	20011012
PRAI US 2001-976535		20011012		

AB Substrates for detecting and measuring the proteolytic activity of botulium type A neurotoxin in an assay are described. Detection is based on an increase in fluorescence due to hydrolysis of these internally quenched fluorescent peptide substrates by botulium type A neurotoxin. Several 13-15 amino acid peptides, derived from the substrate region of **SNAP-25**, have been constructed and analyzed for use in the assay.

RE.CNT 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2002:540137 CAPLUS
DN 137:73251
TI Methods for treating mammary gland disorders

SNARE, vesicle-associated membrane protein-2, reduce SNARE complex formation, H+-ATPase translocation to the apical membrane, and inhibit H+ secretion. The purpose of these experiments was to characterize the physiological role of a second t-SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP)-23, a homologue of the neuronal **SNAP-25**, in regulated exocytosis of H+-ATPase vesicles. Our experiments document that 25-50 nM **botulinum** toxin (Bot) A or E cleaves rat SNAP-23 and thereby reduces immunodetectable and 35S-labeled SNAP-23 by >60% within 60 min. Addition of 25 nM BotE to IMCD homogenates reduces the amount of the 20 S-like SNARE complex that can be immunoprecipitated from the homogenate. Treatment of intact IMCD monolayers with BotE reduces the amount of H+-ATPase translocated to the apical membrane by 52+-2% of control and reduces the rate of H+ secretion by 77+-3% after acute cell acidification. We conclude that SNAP-23 is a **substrate** for **botulinum toxin** proteolysis and has a critical role in the regulation of H+-ATPase exocytosis and H+ secretion in these renal epithelial cells.

L26 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1999:636059 CAPLUS
DN 131:268231
TI Antibody-based assay for **botulinum** and tetanus neurotoxins
IN Shone, Clifford Charles; Hallis, Bassam; James, Benjamin Arthur Frederick; Quinn, Conrad Padraig
PA Microbiological Research Authority, UK
SO U.S., 21 pp., Cont.-in-part of Appl. No. PCT/GB95/01279.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5962637	A	19991005	US 1996-760001	19961203
	WO 9533850	A1	19951214	WO 1995-GB1279	19950602
	W: AU, CA, JP, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 6043042	A	20000328	US 1998-15960	19980130
	US 6337386	B1	20020108	US 2000-534572	20000327
PRAI	GB 1994-11138	A	19940603		
	WO 1995-GB1279	A2	19950602		
	US 1996-760001	A3	19961203		
	US 1998-15960	A1	19980130		

AB The invention provides an antibody-based assay for toxins having peptidase activity, and in particular, this invention relates to assays for **botulinum** and tetanus neurotoxins. The invention comprises the steps of: (a) combining a test compd. with a substrate and with antibody, wherein the substrate has a cleavage site for the toxin and when cleaved by toxin forms a product, and wherein the antibody binds to the product but not to the substrate; and (b) testing for the presence of antibody bound to the product, which product is attached to a solid phase assay component. Preferably, the substrate is a peptide or a protein which is cleaved by the toxin to generate new peptides have N- and C-terminal ends. In addn., the target peptide is preferably selected from the group VAMP, **SNAP-25**, and syntaxin, and it may also be from analogs, isoforms, and/or fragments thereof. Furthermore, the assay is capable of distinguishing between active and inactive toxin present within the sample, since inactive toxin will have reduced or no activity.
RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 9 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4
AN 1997:516389 BIOSIS

DN PREV199799815592
TI Recombinant **SNAP-25** is an effective **substrate** for Clostridium **botulinum** type A **toxin** endopeptidase activity in vitro.
AU Ekong, Theresa A. N.; Feavers, Ian M.; Sesardic, Dorothea (1)
CS (1) Div. Bacteriol., Natl. Inst. Biol. Standards and Control, South Mimms, Potters Bar, Hertfordshire EN6 3QG UK
SO Microbiology (Reading), (1997) Vol. 143, No. 10, pp. 3337-3347. ISSN: 1350-0872.
DT Article
LA English
AB Bacterial neurotoxins are now being used routinely for the treatment of neuromuscular conditions. Alternative assays to replace or to complement in vivo bioassay methods for assessment of the safety and potency of these **botulinum** neurotoxin-based therapeutic products are urgently needed. Advances made in understanding the mode of action of clostridial neurotoxins have provided the basis for the development of alternative mechanism-based assay methods. Thus, the identification of **SNAP-25** (synaptosomal-associated protein of molecular mass 25 kDa) as the intracellular protein target which is selectively cleaved during poisoning by **botulinum** neurotoxin type A (BoNT/A) has enabled the development of a functional in vitro assay for this toxin. Using recombinant DNA methods, a segment of **SNAP-25** (aa residues 134-206) spanning the toxin cleavage site was prepared as a fusion protein to the maltose-binding protein in Escherichia coli. The fusion protein was purified by affinity chromatography and the fragment isolated after cleavage with Factor Xa. Targeted antibodies specific for the N and C termini of **SNAP-25**, as well as the toxin cleavage site, were prepared and used in an immunoassay to demonstrate BoNT/A endopeptidase activity towards recombinant **SNAP-25** substrates. The reaction required low concentrations of reducing agents which were inhibitory at higher concentrations as were metal chelators and some inhibitors of metallopeptidases. The endopeptidase assay has proved to be more sensitive than the mouse bioassay for detection of toxin in therapeutic preparations. A good correlation with results obtained in the in vivo bioassay ($r = 0.95$, $n = 23$) was demonstrated. The endopeptidase assay described here may provide a suitable replacement assay for the estimation of the potency of type A toxin in therapeutic preparations.

L26 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1999:469564 CAPLUS
DN 131:253406
TI Alternative in vitro bioassay methods for BoNT/A activity: implications for potency testing of clinical formulations
AU Ekong, T. A. N.; Feavers, I. M.; McLellan, K.; Sesardic, D.
CS Div. Bacteriol., Natl. Inst. Biol. Stand. Control, South Mimms, Potters Bar, Herts, EN6 3QG, UK
SO Biomedical Aspects of Clostridial Neurotoxins, Conference, Oxford, July 8-11, 1996 (1997), Meeting Date 1996, 142-146. Editor(s): Tranter, Howard S. Publisher: Centre for Applied Microbiology and Research, Salisbury, UK. CODEN: 67WQAB
DT Conference
LA English
AB **Botulinum** neurotoxin type A (BoNT/A) is a powerful toxin which is now being used to treat a no. of neuromuscular conditions. There is a need for replacement assays for toxin activity which do not rely on in vivo methods, indeed, the currently used bioassay is a priority target for replacement. Most in vitro alternative assays are dependent on immunodetection of toxin protein. We have developed a sensitive ELISA assay and shown it to be useful in detecting BoNT/A in clin. formulations. However, there was no relationship between the amt. of toxin detected and its biol. activity as the ratio of active to total toxin varied for

different prepns. This inability to detect active BoNT/A has limited the applicability of ELISAs for potency estns. A valid alternative assay should be mechanism based. The recent discovery that BoNT/A selectively cleaves **SNAP-25**, a synaptosomal assocd. membrane protein, has provided a basis for the development of functional in vitro bioassays. Using recombinant methods we have prepd. a fragment of **SNAP-25** (residues 134-206) spanning the BoNT/A cleavage site as a fusion protein, and have used it as an in vitro **substrate** for the **toxin**. An immunoassay was developed, using targeted antibodies specific for the C-terminus of intact or decleaved **SNAP-25**. The assay was used to examine BoNT/A activity in clin. prepns. It is sensitive, with a limit of detection equiv. to 0.2-0.8 mouse LD50/mL (i.e. <5% of a therapeutic dose), and a gcv of 5.9-11.2% (n = 15). It is simple and rapid, and shows a good correlation with the in vivo bioassay (r = 0.83, n = 31). Preliminary results look promising, but the assay will require further validation before it can be recommended for use in potency estns. of clin. prepns. of BoNT/A.

RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L26 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1996:87093 CAPLUS
DN 124:109558
TI Toxin assay
IN Shone, Clifford Charles; Hallis, Bassam; James, Benjamin Arthur Frederick; Quinn, Conrad Padraig
PA Microbiological Research Authority, UK
SO PCT Int. Appl., 47 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9533850	A1	19951214	WO 1995-GB1279	19950602
	W: AU, CA, JP, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	AU 9526240	A1	19960104	AU 1995-26240	19950602
	AU 687564	B2	19980226		
	EP 763131	A1	19970319	EP 1995-921033	19950602
	EP 763131	B1	19990825		
	R: AT, BE, CH, DE, DK, FR, GB, IT, LI, NL, SE				
	JP 10504801	T2	19980512	JP 1995-500544	19950602
	AT 183779	E	19990915	AT 1995-921033	19950602
	US 5962637	A	19991005	US 1996-760001	19961203
	US 6043042	A	20000328	US 1998-15960	19980130
	US 6337386	B1	20020108	US 2000-534572	20000327
PRAI	GB 1994-11138	A	19940603		
	WO 1995-GB1279	W	19950602		
	US 1996-760001	A3	19961203		
	US 1998-15960	A1	19980130		

AB A **toxin** assay that uses a **substrate** for cleavage by the **toxin** and antibodies that do not recognize the substrate but recognize and bind to the product of cleavage of the **substrate** by the **toxin**. The substrate can be a nerve cell peptide when the assay is for **botulinum** toxin or tetanus toxin.

zinc.
AU Kalandakanond, Sarinee; Coffield, Julie A. (1)
CS (1) Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, GA, 30602: coffield@vet.uga.edu USA
SO Journal of Pharmacology and Experimental Therapeutics, (March, 2001) Vol. 296, No. 3, pp. 980-986. print.
ISSN: 0022-3565.
DT Article
LA English
SL English
AB Previously we reported that **SNAP-25**, synaptobrevin II, and syntaxin I, the intracellular substrates of **botulinum** toxin originally identified in nontarget tissues, were present in a recognized mammalian target tissue, the mouse hemidiaphragm. Furthermore, we reported that **SNAP-25**, syntaxin I, and synaptobrevin II were cleaved by incubation of the intact hemidiaphragm in **botulinum** serotypes A, C, and D, respectively. The objective of the current study was to use the mouse phrenic nerve-hemidiaphragm preparation and **botulinum** serotype A to investigate 1) the relationship of **substrate** cleavage to **toxin**-induced paralysis, and 2) the relevance of **substrate** cleavage to the mechanism of **toxin** action. Immunoblot examination of tissues paralyzed by **botulinum** toxin type A (10-8 M) revealed ltoreq10% loss of **SNAP-25** immunoreactivity at 1 h postparalysis, and gtoreq75% loss at 5 h postparalysis. Triticum vulgaris lectin, an agent that competitively antagonizes toxin binding, antagonized toxin-induced paralysis as well as **SNAP-25** cleavage. Methylamine hydrochloride, an agent that prevents pH-dependent translocation, also antagonized toxin-induced paralysis and **SNAP-25** cleavage. Furthermore, zinc chelation antagonized toxin-induced paralysis and **SNAP-25** cleavage. These results demonstrate that cleavage of **SNAP-25** by **botulinum** serotype A fulfills the requirements of the multistep model of **botulinum** toxin action that includes receptor-mediated endocytosis, pH-dependent translocation, and zinc-dependent proteolysis. Furthermore, the minimal amount of **SNAP-25** cleavage at 1 h postparalysis suggests that inactivation of only a small but functionally important pool of **SNAP-25** is necessary for paralysis.

L26 ANSWER 7 OF 11 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 3
AN 2001:313308 BIOSIS
DN PREV200100313308
TI Role of SNAP-23 in trafficking of H+-ATPase in cultured inner medullary collecting duct cells.
AU Banerjee, Abhijit; Li, Guangmu; Alexander, Edward A.; Schwartz, John H. (1)
CS (1) Evans Biomedical Research Center, 650 Albany St., Boston, MA, 02118-2908: jhsch@bu.edu USA
SO American Journal of Physiology, (April, 2001) Vol. 280, No. 4 Part 1, pp. C775-C781. print.
ISSN: 0002-9513.
DT Article
LA English
SL English
AB The trafficking of H+-ATPase vesicles to the apical membrane of inner medullary collecting duct (IMCD) cells utilizes a mechanism similar to that described in neurosecretory cells involving soluble N-ethylmaleimide-sensitive factor attachment protein target receptor (SNARE) proteins. Regulated exocytosis of these vesicles is associated with the formation of SNARE complexes. Clostridial neurotoxins that specifically cleave the target (t-) SNARE, syntaxin-1, or the vesicle